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THE GERMINATION OF *POA* SPP.

BY ALEXANDER NELSON, B.Sc., N.D.A.
(*Scientific Department, Messrs David Bell, Ltd., Leith.*)

(With 7 Graphs and 2 Text-figures.)

A LARGE proportion of the "seeds" used in agriculture is included in the Natural Order Gramineae and, arising out of the present day insistence on a viability test previous to sowing, the laboratory germination of this class is a problem of importance.

Regarded from the point of view of volume of trade, the genus *Poa* is not of prime importance. Regarded from the point of view of the seed analyst, however, the fickle nature of the species of the genus in germination gives them considerable interest.

The present study was planned, in the first place, to analyse the action of the various factors bearing on germination and correlate them one to the other. The action of various salts was studied, and later, the soil as a substrate was introduced.

Numerous workers have shown that alternation of temperature and exposure to light are aids to germinating "seeds" of the Poas. These two aids are part of the method standard in British laboratories (Saunders (21)). In American laboratories a 1 per cent. solution of potassium nitrate is commonly used in place of tap-water as a source of moisture. The nitrate increases the speed of germination and the total number of seeds germinating.

Material. Samples of "seed" of the four common species (*compressa*, *nemoralis*, *pratensis* and *trivialis*), crop 1924, were obtained and kept under room conditions until February, 1925, when tests were commenced. The purity of the samples to specific character was checked from Hellbo (7). As each test was sown the "seed" to be used was drawn from the sample, care being taken that each individual was made up of a pair of "glumes" and a caryopsis.

Apparatus and Methods. Large, locally made, gas-heated tanks of the Jacobsen pattern, as shown in Fig. 1, were employed. Each 100 of "seeds" was sown on a circular blotting paper pad laid on two blotting

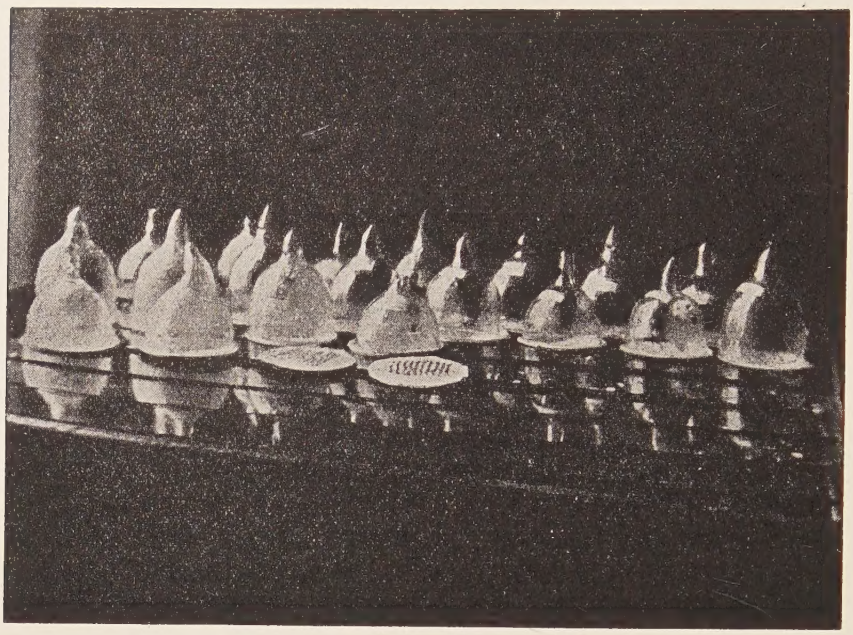


Fig. 1. Tests on germinating tank.

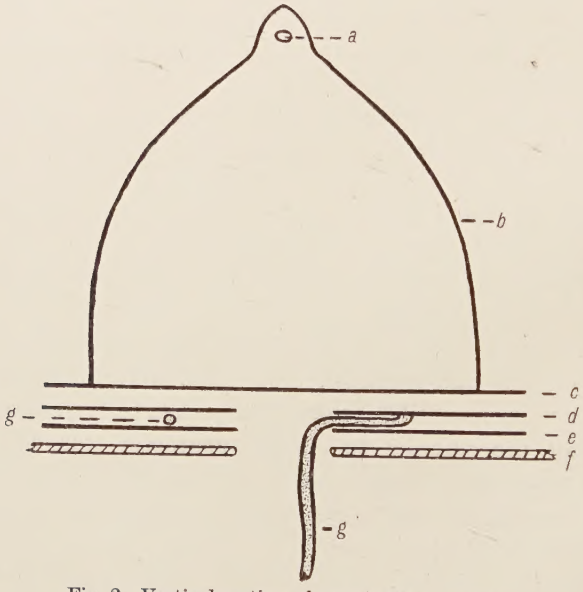


Fig. 2. Vertical section of germinating system.

a, Ventilating hole. *b*, Glass bell-jar. *c*, Blotting-paper "seed-bed" or pad. *d* and *e*, Blotting-paper rings. *f*, Glass bar supporting tests. *g*, Miners' wick.

paper rings. The pads were supported on glass bars running across the tank and kept moist by a length of miners' wick dipping into the tank water. Small clear-glass bell-jars covering the tests prevented excessive evaporation without excluding light (see Fig. 2).

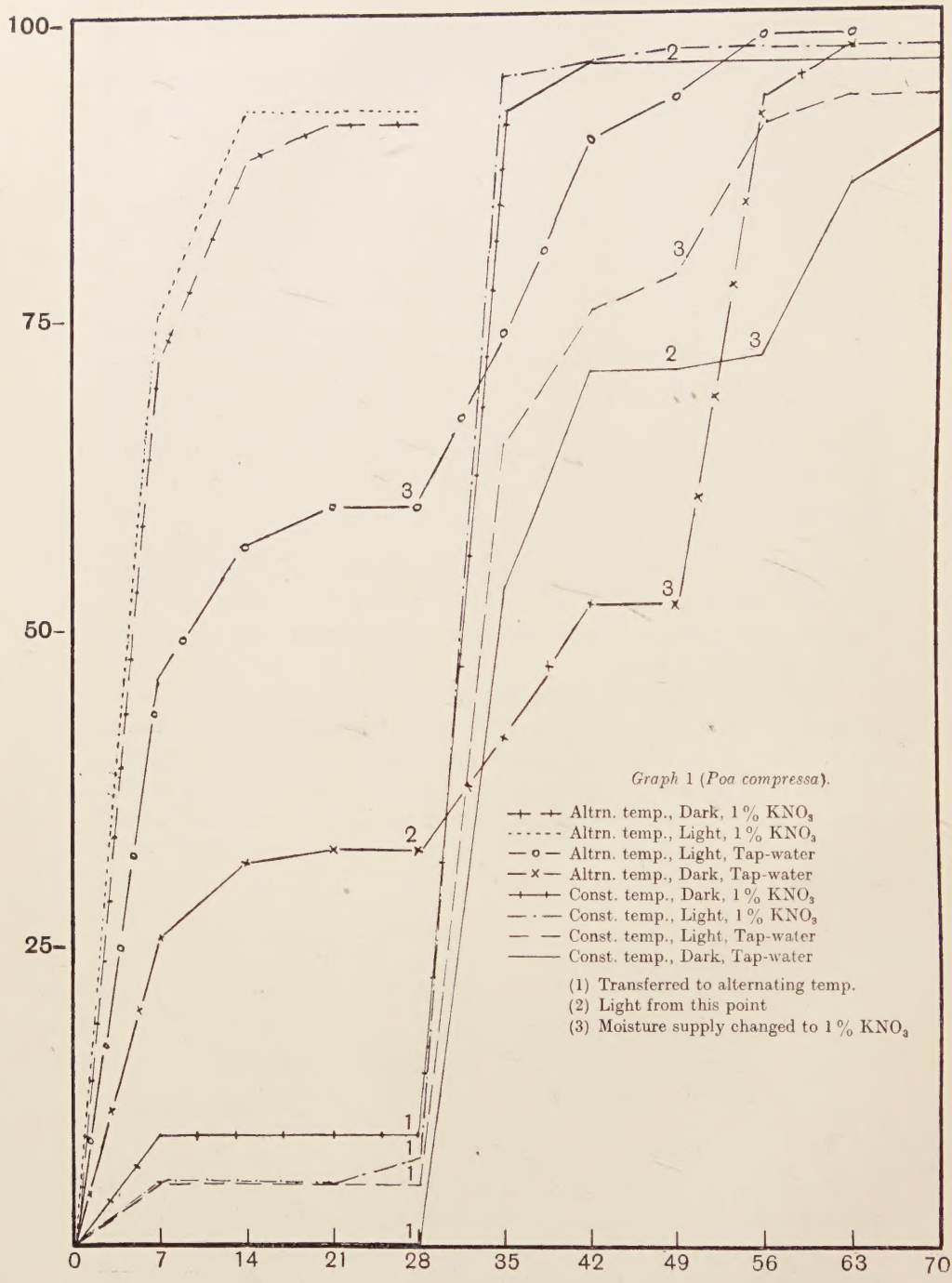
"Constant temperature" indicates that the water in the tank was kept practically steady at 25° C. "Alternating temperature" indicates that the tank water temperature was for 16 hours (5 p.m. to 9 a.m.) at 20°–23° C. and 8 hours at 35°–37° C. The temperature of the seed-bed would be a little lower than the water temperature, the gap depending on the difference between the water temperature and the temperature of the room. Harrington(6) has shown, however, that the alternation is more important than the temperature.

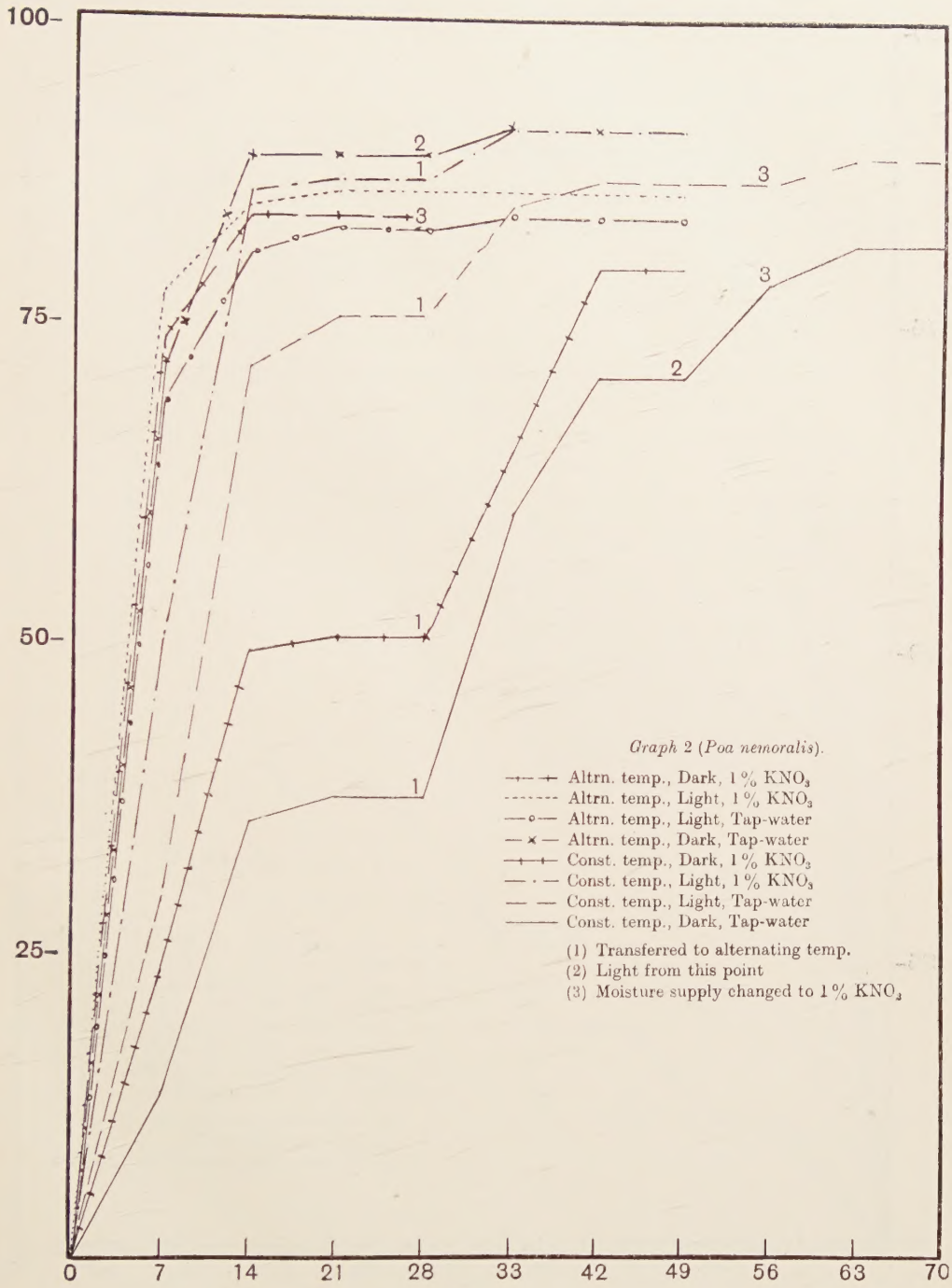
Tests "in the dark" were carried out by substituting brown bell-jars covered with brown paper for the clear-glass ones described above. When any salt solution was to be employed, the pad, rings and wick were moistened with it and the wick allowed to dip into a small jarful resting in the water on the tank bottom. Thus, except when the temperature conditions had to be varied, all tests were situated very close together and received exactly the same conditions except when these were deliberately altered.

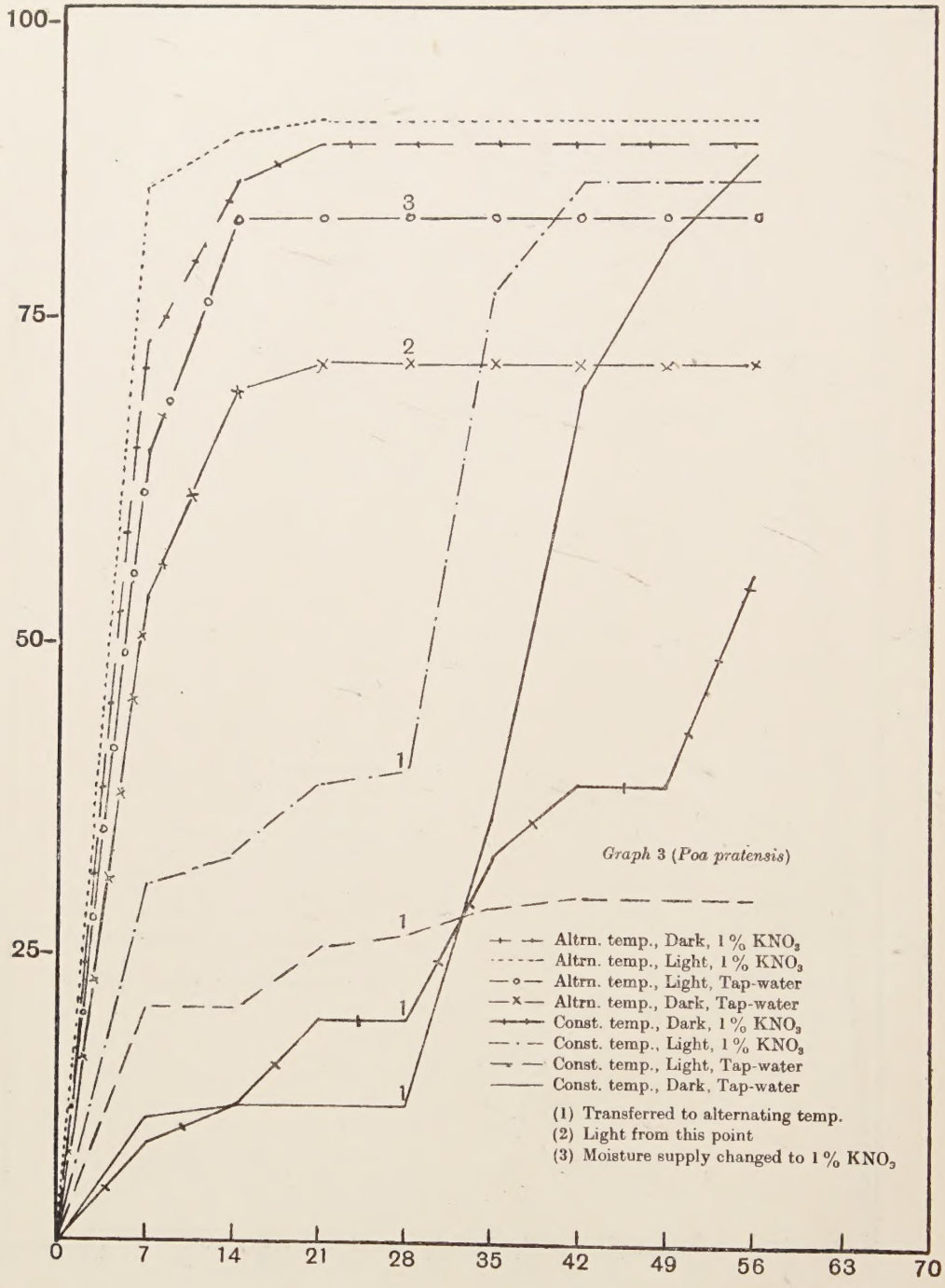
In all the graphs in this paper the period of the test in days is plotted along the base, and the number of "seeds" germinating, on the vertical scale. Every seven days the "seeds" which had germinated were counted off, except in the soil tests where they were counted off from day to day. Each of the four species mentioned above was in turn subjected to test by each of the undernoted methods, a pad carrying 100 "seeds" being allocated to each method.

No.	Temperature	Lighting	Moisture supply
1	Constant temperature	Dark	Tap-water
2	,, ,,	Daylight	,,
3	,, ,,	,,	1 % solution KNO_3
4	,, ,,	Dark	,, ,,
5	Alternating ,,	,,	Tap-water
6	,, ,,	Daylight	,,
7	,, ,,	,,	1 % solution KNO_3
8	,, ,,	Dark	,, ,,

Discussion of Graphs 1–4. Considering for the moment Graph 1, which applies to *Poa compressa*, and dealing only with the first 28 days (the standard period for a routine test), it is seen that the curves form two main groups. The first group (methods 1–4) having been subjected


100–
75–
50–
25–
0
7
14
21
28
35
42
49
56
63
70
1
2
3
Graph 1 (Poa compressa).
Altrn. temp., Dark, 1 % KNO3
Altrn. temp., Light, 1 % KNO3
Altrn. temp., Light, Tap-water
Altrn. temp., Dark, Tap-water
Const. temp., Dark, 1 % KNO3
Const. temp., Light, 1 % KNO3
Const. temp., Light, Tap-water
Const. temp., Dark, Tap-water
(1) Transferred to alternating temp.
(2) Light from this point
(3) Moisture supply changed to 1 % KNO3


100–
75–
50–
25–
0
7
14
21
28
33
42
49
56
63
70
1
2
3
Graph 2 (Poa nemoralis).
Altrn. temp., Dark, 1 % KNO3
Altrn. temp., Light, 1 % KNO3
Altrn. temp., Light, Tap-water
Altrn. temp., Dark, Tap-water
Const. temp., Dark, 1 % KNO3
Const. temp., Light, 1 % KNO3
Const. temp., Light, Tap-water
Const. temp., Dark, Tap-water
(1) Transferred to alternating temp.
(2) Light from this point
(3) Moisture supply changed to 1 % KNO3


100
75
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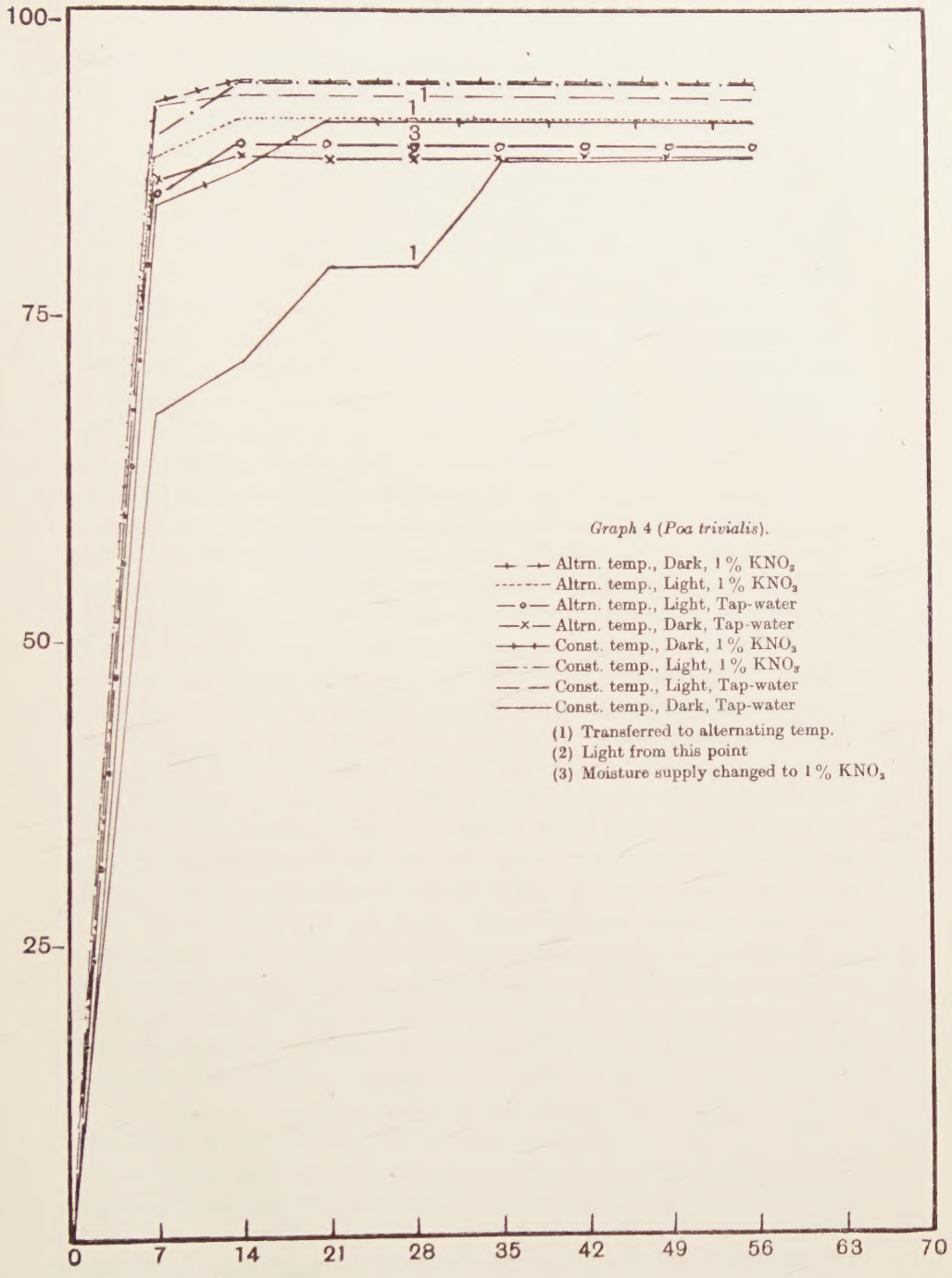
7
14
21
28
35
42
49
56
63
70
3
2
1
1
1
1
1
Graph 3 (Poa pratensis)
Altrn. temp., Dark, 1 % KNO3
Altrn. temp., Light, 1 % KNO3
Altrn. temp., Light, Tap-water
Altrn. temp., Dark, Tap-water
Const. temp., Dark, 1 % KNO3
Const. temp., Light, 1 % KNO3
Const. temp., Light, Tap-water
Const. temp., Dark, Tap-water
(1) Transferred to alternating temp.
(2) Light from this point
(3) Moisture supply changed to 1 % KNO3



Graph 4 (*Poa trivialis*).

Altrn. temp., Dark, 1 % KNO_3
Altrn. temp., Light, 1 % KNO_3
Altrn. temp., Light, Tap-water
Altrn. temp., Dark, Tap-water
Const. temp., Dark, 1 % KNO_3
Const. temp., Light, 1 % KNO_3
Const. temp., Light, Tap-water
Const. temp., Dark, Tap-water

(1) Transferred to alternating temp.
(2) Light from this point
(3) Moisture supply changed to 1 % KNO_3

to constant temperature is of low germination. The second and higher group had alternating temperature. The difference between the two groups is distinct and conclusive. Within each group a certain arrangement manifests itself. At the base of each group lies the pad receiving no stimulation either from light or potassium nitrate. Next in ascending order comes the pad receiving light, then the pads receiving potassium nitrate. Potassium nitrate seems to be effective irrespective of the action of light.

When these tests had run their standard course (28 days) each pad was "stepped up" one place in what had thus been indicated to be the scale of stimulation. Pads 1–4 were now subjected to alternation of temperature: pads 4 and 5 were illuminated: pad 6 was changed to potassium nitrate solution, while pads 7 and 8 were withdrawn. The effect of these changes was immediate and striking, confirming the tentative conclusions based on the first period. The pads of the low group when transferred to alternating temperature responded at once, giving curves similar to their counterparts of the first 28 days, and differing only in that they rose higher—this might be explained by the pre-soaking. Following the curves of the "low group" through, it is seen that they tend to flatten out at various points, but that then the introduction of another "step up" in stimulation produces another rise. The two pads on the alternating temperature group behave in a similar sort of way: "seed" on pad 5 when illuminated at the 28th day immediately recommenced germination and continued until the 42nd day, when it stopped: given nitrate solution at the 49th day "complete germination" resulted. Pad 6, tested by the British standard method (alternation of temperature, light and tap-water), stopped germinating on the 21st day with a total of 60 per cent.: given potassium nitrate on the 28th day it eventually exceeded any other pad.

It is thus seen that *Poa compressa* is very sensitive to conditions of temperature, light and the character of the moisture supply. Alternation of the temperature is of first importance followed by the supply of potassium nitrate. These two together can produce satisfactory results and render the analyst independent of the light supply, but it is better to allow access of light rather than to screen the tests.

Poa nemoralis (Graph 2) appears to be less sensitive to the three factors studied than was *P. compressa*, and further, they seem capable of replacing each other to a greater extent. For instance the pads subjected to alternating temperature all germinated at or about the maximum and the differences between them are not significant.

Therefore, it would appear that alternation of temperature is the only essential factor with this species, light being practically unnecessary and tap-water sufficient. In the "constant temperature group," however, a certain arrangement manifests itself, in that the two pads in the dark (1 and 4) are significantly lower than the two in the light (2 and 3). Further, in either pair the pad receiving KNO_3 is the higher of the two. "Stepping up" the pads produced significant responses, as was the case with *Poa compressa*. It may be concluded from these tests that *Poa nemoralis* will give satisfactory results when any two of the three stimulating factors are operative.

The results obtained with *Poa pratensis* (Graph 3) are somewhat similar to those obtained with *P. nemoralis*, though the effect of alternating temperature appears to be of greater importance and not replaceable by potassium nitrate and light. The behaviour of pad 2 (constant temperature, light and tap-water) is difficult to account for.

Poa trivialis is quite insensible to variations in the conditions as studied, except that the pad lowest in the "scale of stimulation" (constant temperature, dark and tap-water) is significantly lower than the others at the end of the standard period. Further, this deficit is made good by "stepping up," *i.e.* alternating the temperature.

The data offered here do not warrant any extended theoretical discussion of the processes underlying the various effects noted—especially apparent in the case of *Poa compressa*. Each factor evidently has a multiple effect, affecting more than one aspect of the life of the organism or, perhaps more accurately, the activities of the individual cell.

Harrington (6) in reviewing the subject of alternation of temperature mentions a number of hypotheses advanced by earlier workers to account for somewhat similar effects in other species. Briefly these are:

(i) That temperature alternation affects water intake.

(ii) The reserve of materials made available at any given temperature is wholly or largely used up in respiration, but a slight surplus becoming soluble at a higher temperature is available for growth when the temperature is lowered with consequent reduction of intensity of respiration.

(iii) The whole effect of temperature alternation is due to oxygen relationships.

(iv) With alternation of temperature there is more lively gaseous interchange with consequent increase of respiration.

In discussing these with reference to the present study a number of lines of evidence taken from work covering the general flora may be

cited, though their application to germination must be guarded. Hoagland and Davis (8) discussing the absorption of ions by plants from dilute solutions show that light exerts a considerable effect in the case of an alga (*Nitella*). These writers conclude that the absorption of ions by plants from dilute solutions involves energy exchanges, with light as the ultimate source of energy, and they suggest that this absorption is intimately related to growth and metabolism. Jeffs (10) suggested that the light-growth-effect reaction of multicellular organs is not due to increase in cell elongation simply, but to some other effect of light such as change in the rate of cell division. The periodic nature of cell division noted by Kellicott (12) in *Allium cepa*, which probably holds good for many other plants, may be correlated in some way with the germination response to periodic alternation of external conditions and in this connection it is interesting to note that Reed (18) has shown that *Spirogyra* can be stimulated to divide by keeping the filaments at a low temperature in the dark and then transferring to optimum conditions of light and temperature.

That germination in its initial stages, at least, is not growth simply, must be remembered; and, as is well known, conditions affecting growth do not necessarily affect germination with equivalent force but the possible energy effects of light and temperature and their effect on cell division are clearly indicated. Other effects, such as increased permeability, quickened gaseous interchange and altered respiration likewise probably add their quota to the germination responses noted above.

In this discussion and also in what follows it is considered unnecessary to allow for any effect of the plant on the culture medium as reported by Hoagland (9) because of the short period during which the plant is active: growth has proceeded only a short way when the seedlings are counted off.

Turning to consideration of the stimulation resulting from the use of potassium nitrate it would again appear that the effect is complex. Most of the published work connected with this aspect deals with tissues from the mature plant or at least with the plant long after germination.

Nobbe, Shröder and Erdman (16) indicate that potassium has an effect on the translocation of starch. Copeland (2) states that potassium is a direct factor in increasing the turgor of the cell. Purvis (17), working with *Dactylis*, concludes that potassium salts influence the physiological condition of the plant or its chemical composition. Macallum (13) has shown that potassium is always found where new outgrowths are in

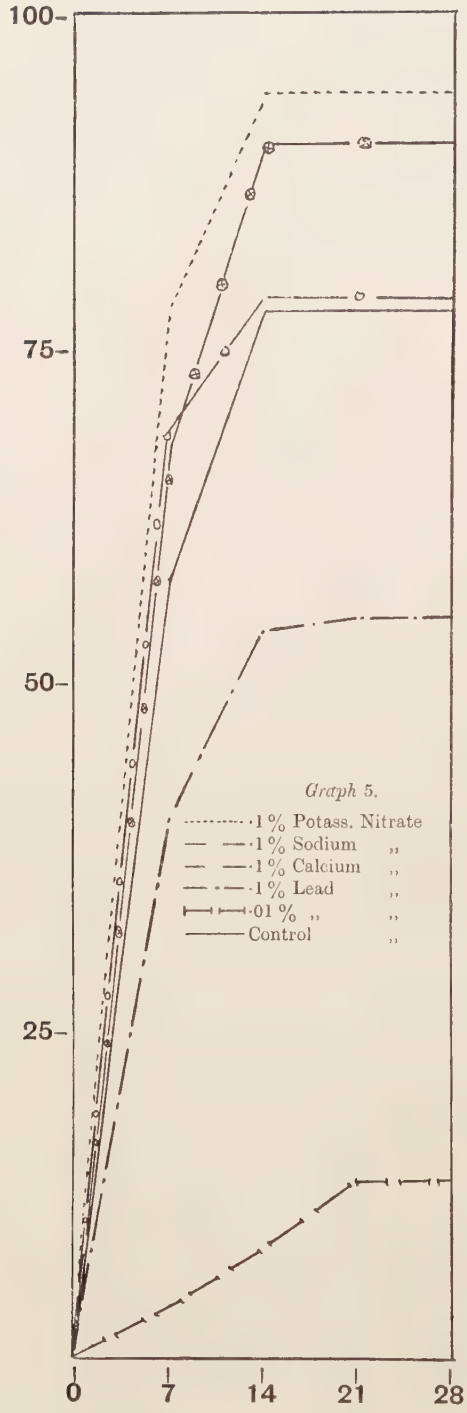
process of formation, while Dowding(4) concludes that potassium is essential to all meristematic tissue. Reed(18) has shown that potassium is necessary for the germination of moss spores. This general and widespread connection between potassium and actively developing tissue, coupled with the fact that Reed(18) found potassium essential for mitotic division, leads to the conclusion that potassium affects the dividing nucleus in a favourable way. Another mode of action for potassium may be to stimulate respiration as noted by Matthews(14) who also says, "The general richness of potassium in cells of widely different character indicates that this element must be concerned with some fundamental process or condition in the cell, and it is possible that that process is respiration. But just why it is favourable and what its real function is it is impossible to state."

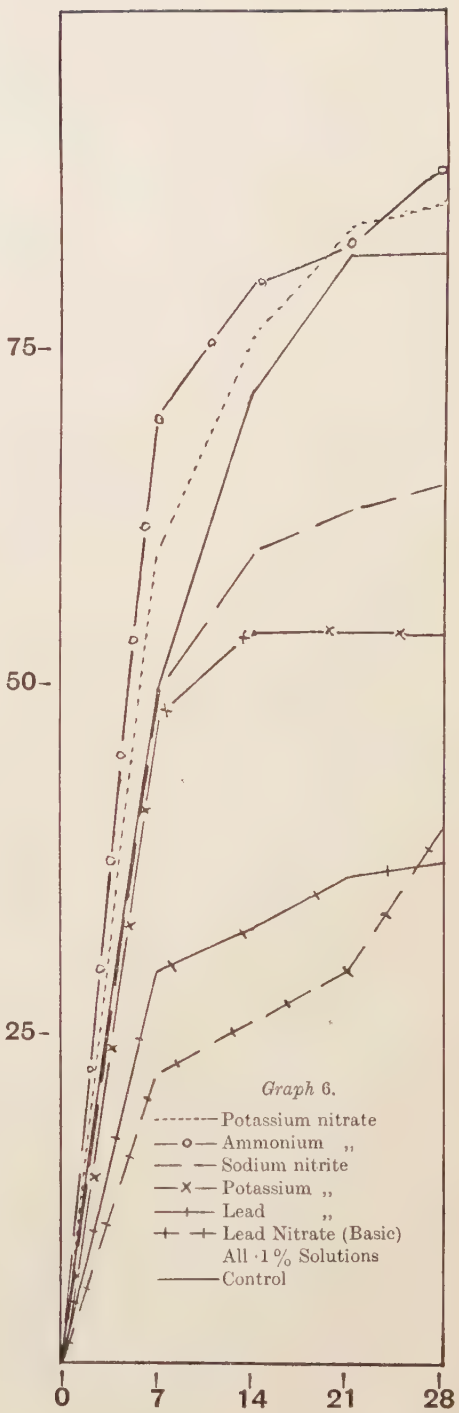
To sum up, it seems reasonable to believe that each of these three factors studied has a number of modes of action, the value of these depending on the species in question and the condition of the seed at the time of the test. Their relation to the question of increased or accelerated nuclear division, though not previously considered in connection with germination effects, is undoubtedly of some considerable significance.

FURTHER WORK IN THE LABORATORY.

The next step was to compare the action of various salts in solution when used as the moisture supply. All this work was done on the sample of *Poa compressa* used for the tests shown on Graph 1. The salts selected included the nitrates commonly used as manures in general agriculture, *i.e.* calcium and sodium nitrates. Lead nitrate which Berry(1) found stimulating to the growth of oat *plants* was also included. All the tests shown on either Graph 5 or 6 were run simultaneously.

For Graph 5, 0·1 per cent. solutions (except one pad of lead nitrate) were employed and the results indicate that in promoting germination nitrates of potassium, sodium and calcium are superior to the control (tap-water) in the order named. Lead nitrate proved to be distinctly depressant, though of the two concentrations used the lower one had less effect. It was very interesting to note in this and all other tests with lead nitrate in concentrations such as these, that the glumes of the "seeds" all expanded and the appearance of the test at an early stage promised a high result. The concentrations used are very much higher than those employed by Berry. Later tests (when the seed may have altered in character) with a graduated series of concentrations in


100
75
50
25
0
7
14
21
28
Graph 5.
·1 % Potass. Nitrate
·1 % Sodium ,,
·1 % Calcium ,,
·1 % Lead ,,
·01 % ,, ,,
Control ,,


75
50
25
0
7
14
21
28
Graph 6.
Potassium nitrate
Ammonium ,,
Sodium nitrite
Potassium ,,
Lead ,,
Lead Nitrate (Basic)
All ·1 % Solutions
Control

light and dark indicated that at no strength was a solution of lead nitrate stimulating to germination.

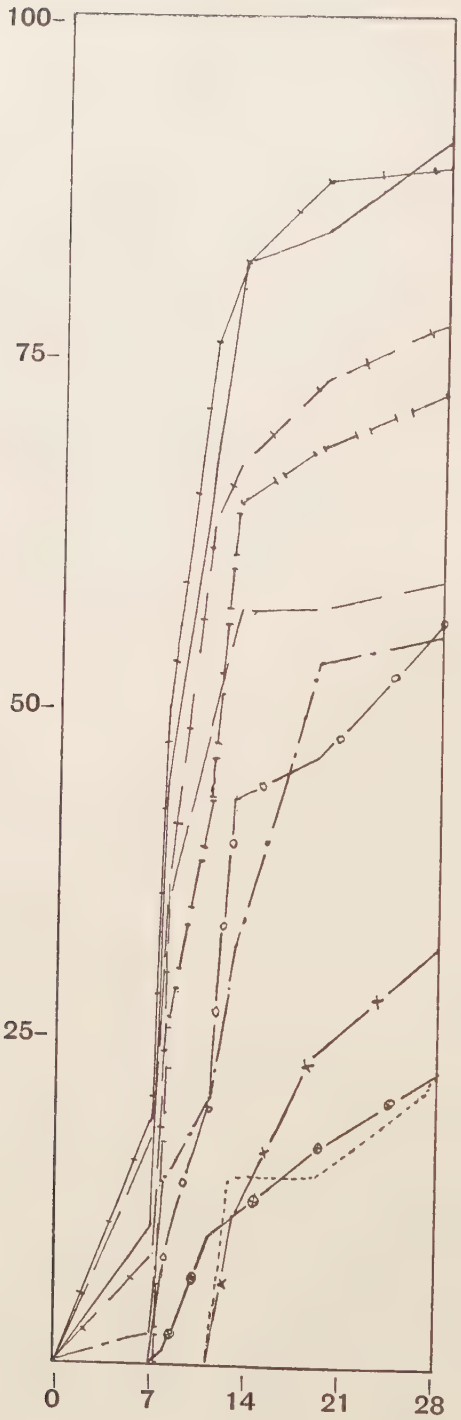
In Graph 6, again, a number of salts are compared. Ammonium nitrate was found to be at least equal to potassium nitrate in stimulating effect. Sodium and potassium nitrites were depressant while the lead salts, nitrite and basic nitrate, were also extremely depressant. The relative positions of sodium and potassium nitrites are interesting as they are the reverse of the nitrates of these bases, and suggest that the ions must not be considered alone but together, though the different effect of the different products following on ionisation must not be forgotten. Stiles (22) and a number of observers mentioned by him have shown that potassium is more rapidly absorbed by plant tissue than sodium, and either of them more rapidly than calcium: this is the order in which the nitrates of these bases fall when arranged according to their stimulative effect on the germination of *Poa compressa*. The effect of lead salts in proportion to their concentration is in agreement with the findings of Dilling (3) who noted the effect of lead acetate on cress and mustard seedlings.

SOIL TESTS.

The same sample of *Poa compressa* was used for the soil tests as was employed in the laboratory tests.

Method. Small pots (60's) were filled with some 150 gm. of air-dried potting soil passed over a No. 6 sieve. 0·1 gm. of salt was dissolved in 100 c.c. of tap-water and this used to saturate the soil, one salt being allocated to each pot. The surface of the soil after mixing was lightly pressed level and the seed sown one by one, each being gently pressed into the surface. Williams (23) has shown that for *Poa pratensis*, at least, the seed should not be buried.

In the tests shown on Graph 7, in addition to the pots used for the seven salts experimented with, three pots were included which had been moistened with tap-water only—these were included to try the effect of different degrees of lighting. Each pot was covered with a small square of 21 oz. glass and over all a sheet of "glassene" paper was spread, except over one of the tap-water pots. The seed in this pot was thus exposed to the full glare of the sun, the curve of this test being designated "sunlight." One of the remaining tap-water pots had its glass cover painted over with black enamel: this pot provided the "dark" results. The tests were conducted in a greenhouse in the south-east of England, the sunlight being brilliant and the temperature as



Graph 7.
Soil tests.

- Sunlight
- Shaded daylight
- Dark
- Lead nitrate
- Lead ,, (Basic)
- Lead nitrite
- Calcium nitrate
- Ammonium ,,
- Sodium ,,
- Potassium ,,

Nitrites of sodium and potassium gave no germination

read on the maximum-minimum thermometer very much like the alternation produced in the laboratory. "Seeds" germinating were counted off from day to day and the tests ran for 28 days. As the soil in the pots dried it was moistened with its appropriate solution by sub-irrigation. It is of interest to note that the "dark" pot and the pots moistened with nitrites (all bases) did not dry so quickly as the others, this difference between the pots being very striking.

Consideration of the results plotted in Graph 7 shows that of the three pots moistened with tap-water the one in shaded daylight is very significantly higher than those in "dark" or "sunlight." This indicates that the pots moistened with salt solutions were in the best light conditions.

Morada (15) has shown that exposure to full sunlight for the whole period available per day has a depressing effect on the germination of *Carica papaya*, though exposure for a short time proved beneficial. Dark, he found, induced dormancy.

Considering the tests where salt solutions were employed, the very striking fact emerges that the salts found to be stimulating in laboratory water culture proved to be the most depressant in the soil, while the salt most depressant in water culture was the only one which equalled or exceeded the best control (tap-water). As has been noted, all the nitrite pots held the moisture much better than the others, but though the lead nitrite pot produced the largest number of plantlets the nitrites of sodium and potassium produced not one. Another point of interest was that the surface of the soil in all the pots excepting the "dark" and the "sunlight" pots and those moistened by sodium and potassium nitrite was covered in varying degree with a green alga. The lead nitrite pot had easily the heaviest growth of alga. Thus, while it may be that some of the stimulating effect of the lead nitrite on the seed was due to the better moisture conditions, it must be noted that the dark pot and the sodium and potassium nitrite pots provided equally good physical conditions but very much lower biological results.

Why there should be such a complete reversal of germination effect between the two groups of salts is difficult to explain, but the results certainly confirm the conclusion of Jensen (11) who worked with wheat seedlings. Discussing the toxic effect of salts, that writer concludes that it does not follow that because a salt is highly toxic in solution it is equally so in the soil, nor that one which holds a relatively high position in the toxic table of the soil should occupy the same relative position in solution cultures. It would appear too, from the results here

presented, that the same sort of conclusion is equally true of the stimulating effect.

Berry (1) noted in growth trials with oats that lead salts induced fleshier, broader and darker coloured leafage: this too was clearly seen in the tests reported here, the first leaves of the seeds which germinated on the lead pots being decidedly darker in colour, broader and more fleshy than those on the non-lead pots.

General Discussion.

All the results reported here bear on several practical problems, more particularly on such questions as to how far laboratory tests are referable to soil conditions, and also in what way artificial manures may affect germination in the field. With regard to the first question it is interesting to note that the soil test in "shaded daylight" compares favourably with any laboratory test, though it must be remembered this pot would be under ideal conditions not likely to occur in the open field. With regard to the second question it is indicated that none of the manurial salts commonly employed in agriculture should be applied while grass "seed" is germinating. While the average concentration in the field would never be as high as those used in these tests, owing to the irregular distribution of the manure, certain areas would undoubtedly reach the concentrations here described and so, possibly, lead to a "patchy braid." These results obtained with *Poa compressa* may not apply absolutely to other grasses more commonly employed in agriculture.

The effect of lead salts on the growth of the alga and the physiological effect evidenced by the deep coloration accompanied by increased fleshiness of the grass leaf requires further study but of peculiar interest are the results obtained by Reznikoff (19) working with *Amoeba*. This worker using $PbCl_2$ found two effects, one on the cell surface he ascribed to an effect of the acid formed following on ionisation of the salt. A second and more fundamental effect occurring within the protoplasm he ascribed to the cation. Only the first mentioned of these two effects was lethal. Reznikoff suggests that lead within the cell reacts with the phosphates and carbonates. This may be the mechanism resulting in the leaf effect described above.

SUMMARY.

The paper in its first part deals with the germination response, in the laboratory, of four species of *Poa* to variations of temperature, light and the nature of the moisture supply. The second portion deals with the same response of *P. compressa* to solutions of various salts used as moisture supply. The third part deals with the germination of *P. compressa* in the soil when all the above factors were varied. In the laboratory, alternation of temperature proved to be, generally, the most efficient stimulant followed by light and a solution of potassium nitrate as moisture supply. *P. compressa* proved to be more sensitive than any of the other species considered, *P. pratensis* and *P. nemoralis* rather less so, while *P. trivialis* was almost insensible to variations of the conditions. In the soil, excess of light (sunlight) proved to be depressant. Some salts, *e.g.* potassium nitrate, sodium nitrate, ammonium nitrate, etc., proved to be stimulating in solution culture, while salts of lead were depressant. As a general statement these salts, found to be stimulating in solution culture, proved to be more depressant in soil than those found depressant in the solution. Lead salts in the soil produced broader, deeper coloured leafage than the controls. Nitrites had a striking physical effect in preventing the drying up of the soil.

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THE TRANSMISSION OF "MOSAIC" DISEASE IN HOPS BY MEANS OF GRAFTING

By T. C. THRUPP, B.Sc., A.R.C.Sc.

(With Plate XI.)

Introductory.

The characteristic symptoms of the "mosaic" disease of hops are, mottled green and yellow areas in the leaves (called "mosaic" mottling), the curling in of the edges (recurved), and a noticeable brittleness of the leaves and petioles and of the tips of the bines. The yellow areas are translucent and are clearly visible when a white paper sheet is placed a few inches behind the leaves, or when these are viewed against the sky. The earliest symptoms of an attack are "mosaic" mottling and abnormal shape of the newer leaves of some of the laterals, while the bines themselves may have grown to their full length, produced hops, and have apparently quite normal leaves. In the following season, in addition to all the characteristic symptoms of the disease, the bines fail to grow to their normal length, fall away from the strings or the poles, die back at their tips, and produce few or no hops. In the final stage of the disease, in the same or the following season, the whole plant dies. Occasionally one hop "hill," originally planted with one hop "set[1]," produces both "mosiac" and healthy bines. "Mosaic" disease occurs in many hop gardens in this country and frequently causes serious losses; it necessitates prompt grubbing of infected "hills" to prevent its rapid spread. An account of the disease from the economic point of view has been given by Prof. E. S. Salmon (2).

As no evidence has been produced to show that any visible organism is associated with the disease, it has been generally assumed that the cause must be a "virus." Miss Lacey (1) has shown that protozoa are not present in the phloem of affected hops. Preliminary experiments were carried out at the South-Eastern Agricultural College, Wye, in which the expressed sap of diseased hops was injected into healthy hops (5)

[1] In commercial practice more than one "set" or cutting is frequently planted to the "hill."

but no positive results were obtained. In the case of certain "virus" diseases of plants, grafting has been found a necessary means of investigation; this method was practised in 1925 by Salmon and Ware (5), with the primary object of determining whether a tender and succulent plant such as the hop would submit to such treatment. It was shown that grafting as a process was feasible, but no work with "mosaic" affected material was carried out. From April 1926 and onwards to July grafting was used in the investigations described in the present paper, both for determining whether the disease could be transmitted by grafting, and in order to investigate a further and subsidiary problem which had arisen as far back as 1922. In that year, reports had been received from hop growers (see (4), p. 12) that a new variety of hop—"M 45"—bred at Wye by Prof. Salmon, which had never shown symptoms of "mosaic" disease, appeared to be infecting neighbouring hops of commercial varieties and might, therefore, prove to be a "carrier."

Experimental.

Material was available at Wye, both in the experimental hop garden and in a stock of specially collected plants grown in pots in a greenhouse, and in the hop gardens at the East Malling Research Station. During a part of March and in April a number of trial grafts were made in the hop garden at Wye, in order to gain experience in the process of grafting. In this trial grafting and in the experiments themselves, the stock bine was cut across with a clean safety razor blade, and a V-notch cut in that part of it left on the plant. The selected tip was placed on a clean glass slip and cut to fit the notch. The wedge-shaped end of the tip was then inserted in the V-notch and bound in place with a thin rubber strip, the last turn being fastened with rubber solution. In some of the practice grafts, female scions were grafted on to male stocks; in one case a male scion was grafted on to a female stock; in several cases double grafts were made by grafting one female scion on to a short piece of bine from another female plant, while this again was grafted on to a male stock. In most cases, and also throughout the experiments, the graft was made about 5 cm. above the soil and the scions were about 15 cm. long. Being grafted early in the season the scions of trial grafts grew to the top of the wire work (about 14 ft. from the ground) and in due course produced a normal crop of hops. The male scion also grew well and flowered. In one case a lateral from the middle section of a double graft grew to the top of the wire work and also produced a good crop of hops. This preliminary work showed that grafted healthy hops

will grow as well as normal ungrafted bines, provided the grafting is carried out sufficiently early in the season.

When using healthy material 87·5 per cent. of grafts succeeded, with diseased stocks the percentage successful was only 14·3. The grafting experiments, which began at the end of May, are classified below according to the problems in view.

Upward passage of the supposed virus from "mosaic" stock. Ninety-four healthy susceptible tips were grafted on to "mosaic" stocks. Of these grafts 16 "took" and grew. The scions were as follows: (*a*) commercial varieties of hops known to be susceptible to the disease, viz. Tutsham (7), Cooper's White (2), Eastwell Golding (1), Farnham Whitebine (1), Cobb's Golding (1); (*b*) male hops (2); (*c*) the seedling variety M 45 (2). All the scions of the commercial varieties (with the exception of the Farnham Whitebine[1]) developed "mosaic" symptoms. In view of its known history, it was not expected that the two scions of M 45 would develop the "mosaic" symptoms. It was, however, surprising to find that one of these throve to such an extent that it grew to a height of about 4·5 metres and remained free from all symptoms of the disease, while the remaining bines of the "mosaic" stock were nearly all dead from the severity of the attack. The two male hop scions grew and showed no "mosaic" symptoms, but in these cases the stock was not as badly attacked by the disease. One of these male hops was a plant growing at the Research Station, East Malling (hill 12 in the row of the Tutsham variety); the other was a "wild" hop (Ref. No. A). It is possible that these male hops may be immune; Prof. Salmon has reported (3) the existence of male hops immune to the "mosaic" disease.

A typical case of a susceptible scion on a "mosaic" stock may be given here. A Tutsham scion was grafted on to a "mosaic" stock on June 20th. All the shoots of the stock were affected with true "mosaic" disease. The grafted bine grew to about one metre in length without exhibiting any noticeable symptoms of the disease, but on August 3rd some of the primary leaves showed "mosaic" mottling. On August 16th

[1] The Farnham Whitebine scion lost its growing point by accident, and the lateral which took its place grew very slowly. In consequence the bine was only about 0·75 metre long by September 11th. Among the grafts made out of doors, it was found that the checks in growth were correlated with less intense "mosaic" symptoms when these did develop. Further, it was noticed in several cases that if the symptoms did not appear until September they were less definite than usually observed. In the case of this Farnham Whitebine scion, the stock had both "mosaic" and healthy bines at the time of grafting, and the healthy bines remained free from "mosaic" symptoms to the end of the season. In view of the above facts, the negative result obtained must not be unduly stressed.

the mottling was much more pronounced and most of the leaves curled downwards. By the end of August all primary leaves showed the characteristic symptoms of the disease, and the bine was indistinguishable in appearance from the "mosaic" bines of the stock. The grafted bine had grown to a length of 1·25 metres by the end of the season. The scion was obtained from a healthy plant growing in a pot, the remaining shoots of which were free from the disease throughout the season.

Upward passage of the supposed virus from "carrier" stock. Forty-four grafts were made, using the variety M 45 as stock. Of the 26 grafts which "took," the scions were of the following varieties: Tutsham (7), Eastwell Golding (5), wild hop "C" (3), Farnham Whitebine (2), wild hop "B" (5), wild hop "D" (1), wild hop "E" (1), and 2 male hops. The first 17 cases developed "mosaic" symptoms, while the other 9 cases did not do so. It was noticeable that grafts made in the greenhouse on "carrier" stock did not develop the curling of the leaf edges in the same manner; generally speaking the curling in was on the upper surface, but not in so marked a manner as the curling in on the lower surface observed with the outdoor grafts. The "mosaic" mottling was quite distinct. The highest temperature recorded in the greenhouse was 33° C.

The details of a typical case when using a susceptible scion may be given. A Tutsham tip was grafted on to an M 45 stock in the hop garden at Wye on April 19th. This scion grew and appeared normal and healthy until June 19th, when the primary leaves were seen to have "mosaic" mottling and their edges to be slightly curled downwards; the bine (graft) was then about 1 metre long. On August 20th it had grown to 1·79 metres, the primary leaves were much more mottled and the curling was more pronounced. Subsequently the leaves of the laterals grew out and all developed "mosaic" mottling with a certain amount of curling, those at the tips having abnormal outlines. This bine is lettered *C* in the photograph reproduced in the Plate.

Controls. As regards controls, all the parent hop plants, with one exception[1], from which tips had been taken to graft on to "mosaic" and "carrier" stock, remained free from all symptoms of the disease throughout the season. As additional controls 24 single grafts of healthy susceptible scions were made on healthy susceptible stocks, and 7 double grafts were made from similar material. Of the single grafts 21 "took,"

[1] The experiment in which this control plant was used was discarded and is not included in the number 91, given in Table I.

the hops used being: Tutsham (9), Eastwell Golding (3), wild hop "B" (2), wild hop "C" (3), wild hop "D" (1), and wild hop "E" (3); in each case the stock used was of the same variety as the scion; all grew well and the majority produced hops. All remained free from all "mosaic" symptoms. Of the 7 double grafts, which were made in the manner already described for the practice grafting (*but* with all the component parts of the same variety), 6 were successful: Tutsham (2), wild hop "B" (2), wild hop "C" (1), and Eastwell Golding (1); all grew fairly well and none showed "mosaic" symptoms at any time. Similarly 5 "carrier" scions were grafted on to "carrier" stock, without inducing any "mosaic" symptoms to appear; two even reached a height of 14 ft. and produced a good crop of hops.

No special precautions were taken against insects during these experiments, because it was considered that grafting would give decided results in a very short time and because adequate controls were provided by leaving as many bines as possible on the hill which served as stock, and by keeping under observation all parent plants from which any scions were taken. A summary of the grafting experiments is given in Table I.

Table I.

Summary of "mosaic," healthy and "carrier" grafting experiments.

	Scions				
		Number of healthy			
Stocks	Totals	Taken	Failed	Positive result	Negative result
"Mosaic"	91*	13	78	10	3†
Healthy	24	21	3	0	21
"Carrier"	44	26	18	17	9‡
		Number of "carrier"			
"Mosaic"	2	2	0	0	2§
Healthy	—	—	—	—	—
"Carrier"	5	5	0	0	5

* See footnote on p. 178.

† Of these three scions which took, and from which negative results were obtained, two were from male hops which may perhaps be truly immune, and one was from the Farnham Whitebine which has already been discussed in detail (see p. 177).

‡ The origin of these nine scions was as follows: wild hop "B" (5), wild hop "D" (1), wild hop "E" (1), and the two male hops mentioned above at p. 178. The consistent negative results with respect to wild hop "B" may perhaps be regarded as evidence of immunity and the same may possibly be true also with respect to the others.

§ It was not expected that M 45 scions grafted on to "mosaic" stocks would give positive results (see above, p. 177).

The writer desires to express his indebtedness to Prof. E. S. Salmon, F.L.S., and to Mr W. M. Ware, M.Sc., for the benefit of their frequent advice given during these experiments.

Thanks are also due to the Director of the East Malling Research Station, and members of his Staff, for the use of material and for help given in the course of the work.

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SUMMARY.

1. In these experiments, where susceptible scions have been grafted on to "mosaic" stock, all developed "mosaic" symptoms with one exception. Circumstances were not favourable in this particular case and it should be regarded as giving indecisive not negative evidence. From the other cases there is satisfactory proof that the supposed "virus" can be transmitted up the growing bine.

2. With the "carrier" experiments, all the known susceptible scions, which were grafted on to the "carrier" (M 45) stock, exhibited "mosaic" symptoms. The variety M 45 must, therefore, be regarded as capable of transmitting the disease to susceptible hops, and as being a "carrier."

3. There is no evidence that the process of grafting can of itself in any way induce "mosaic" symptoms to appear in healthy susceptible hops, or in the "carrier" M 45.

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EXPLANATION OF PLATE XI.

Four grafts made on "carrier" stock, photographed July 12th, 1926.

A, Normal ungrafted M 45 bine, showing healthy foliage.
B, Wild hop "B," grafted April 23rd, 1926, showing healthy foliage.
C, Tutsham, grafted April 19th, 1926, showing down-curled "mosaic" affected leaves.
D, Tutsham, grafted May 5th, 1926, showing well-marked "mosaic" symptoms.
E, Eastwell Golding, grafted May 11th, 1926, showing weaker growth and evident but less pronounced "mosaic" symptoms.

0·175 nat. size.

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THRUPP.—Transmission of "Mosaic" Disease in Hops by means of Grafting (pp. 175—180).

IMMUNITY OF POTATO VARIETIES FROM ATTACK BY THE WART DISEASE FUNGUS, *SYNCHYTRIUM ENDOBIOTICUM* (SCHILB.) PERC.

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(With Plates XII and XIII.)

It is a common horticultural practice to graft the shoot system of one plant on the root system of another. Since the grafted plant thus produced usually grows, it follows that the scion must be dependent on the stock for its mineral salts, and the stock on the scion for the substances necessary for its growth. Certain chemical compounds therefore must pass freely between stock and scion across the graft fusion layer.

These substances sometimes become changed after passing through this layer. Colin and Riolle (3), Colin (4, 5), and Colin and Franquet (6) proved that when the artichoke (*Helianthus tuberosus*) is grafted on the sunflower (*H. annuus*), the inulin, a carbohydrate which is characteristic of the artichoke, on passing into the sunflower, is rapidly hydrolysed and changed to saccharose.

Other compounds have been proved to remain unchanged after having traversed the fusion layer. Belladonna plants contain atropine and normal tomato and potato plants do not, but from the work of Laurent (15, 16) and Javillier (11) it is evident that when parts of Belladonna and tomato plants are grafted together, an alkaloid similar to, if not identical with, atropine is sometimes found in small quantities in the tomato tissues. Further, Javillier (11) grafted together portions of Belladonna and potato plants and found, both by chemical and physiological tests, that certain parts of the grafted potato tissue contained a substance apparently identical with atropine. Again, Grafe and Linsbauer (9), on grafting together pieces of *Nicotiana tabacum* and *N. affinis*, found that the amount of nicotine in the *N. affinis* part became increased from nil,

or practically nil (*N. affinis* used in their experiments contained no demonstrable quantities of nicotine), to as much as 1·67 per cent. on dry weight.

Brown (2) has shown that the mineral composition of the fruit of a given variety of apple varies according to the stock on which the variety is grafted. That grafting a scion on a stock may affect more than the mineral composition of that scion is suggested by the work of Barker and Grove (1), who found that the acidity, and probably the tannin content, of apple juice is affected by the type of stock on which the apple is grafted.

Without going further into the literature of the highly controversial subject of the effect of stock on scion and of scion on stock, it is sufficiently clear, firstly, that a stock may cause a change in the mineral composition as well as in other properties of the scion grafted on it, such as acidity, essential oil content (Daniel and Ripert (8)), and probably tannin content; and secondly, that a shoot system may cause changes in the chemical composition of the stock on which it is grafted through the permeation of the tissues of the stock by substances built up in the scion.

Grafting, therefore, offers a method for determining whether a given plant character, such, for example, as immunity, is innate in the cell, or whether it is conditioned by substances which can travel freely about the plant; if the character is of the second kind the contributions of the various tissue systems, roots, foliage, etc., may be determined.

The present paper describes an attempt to investigate, by the method of grafting, the general nature of the immunity of certain varieties of potatoes from attack by the fungus causing Wart Disease, *Synchytrium endobioticum* (Schilb.) Perc.

For our present purpose the potato plant may be looked upon as being composed of three physiological units:

(1) The root system, which supplies the plant with mineral salts and water.

(2) The shoot system, the photosynthetic unit.

(3) The tuber, or storage unit.

The problem resolves itself into building up, by grafting, composite plants of all possible types, and testing the tubers for any resulting effect in regard to immunity from, or susceptibility to, the disease. The following eight types are possible.

Reference will be made to these types both by number and by "formula."

Type	Root	Shoot	Tuber	Formula $\left(\frac{\text{Shoot}}{\text{Root}}/\text{Tuber}\right)$
1	I	S	I	$\frac{S}{I}/I$
2	S	I	S	$\frac{I}{S}/S$
3	S	I	I	$\frac{I}{S}/I$
4	I	S	S	$\frac{S}{I}/S$
5	S	S	I	$\frac{S}{S}/I$
6	I	I	S	$\frac{I}{I}/S$
7	S	S	S	$\frac{S}{S}/S$
8	I	I	I	$\frac{I}{I}/I$

S = susceptible, I = immune.

EXPERIMENTAL WORK.

Grafts of types 1 $\left(\frac{S}{I}/I\right)$, 2 $\left(\frac{I}{S}/S\right)$, 7 $\left(\frac{S}{S}/S\right)$ and 8 $\left(\frac{I}{I}/I\right)$ were dealt with in an earlier paper, Roach (17). In the experiments then reported it was found that shoots of susceptible and immune varieties respectively used as scions did not change the immunity or susceptibility of the tubers produced on the stocks.

In the experiments in question the effect of the grafted-on foliage was tested during the following season by growing the tubers produced by the grafted plant in contaminated soil. It might be objected that by this method any immunity-conferring or susceptibility-bestowing substance stored in the tuber would be "diluted" when the tuber gave rise to a new potato plant, and that the concentration of the substance might thus be insufficient to produce its normal effect. To meet this objection a few grafted plants were grown in contaminated soil, and the effect on the developing tuber was watched, instead of that on the plant arising from this tuber in the following season, as in the previous experiments. Again, however, no effect on the grafted-on foliage on the immunity or susceptibility of the tuber was detected. Confirmation of these results comes from the work of Köhler (14), who grew his grafted plants in contaminated soil, and sought the effect of grafting in the tubers as they developed on the actual grafted plant.

Grafts of types 3 $\left(\frac{I}{S}/I\right)$ *and* 4 $\left(\frac{S}{I}/S\right)$. To build up plants of these types in order to test the effect of a "foreign" root system on the

behaviour of the resulting tuber towards wart disease, grafts of the same kinds as those used for types 1 and 2 were first made. The soil in which the grafted plant was growing was washed away until the whole of the stalk was uncovered and exposed to light. A shallow trough was then built up around the stalk just above the level of the graft union and filled to a depth of 3 or 4 in. with dry sand. In this way the formation of tubers above the graft was induced, but root formation was prevented.

In experiment No. 519 Majestic (I)[1] was grafted as scion on Arran Chief (S)[2]. Three full-sized tubers were produced above the graft. All attempts to alter the behaviour towards wart disease of either the tubers or the leaves of the plants above the graft union failed. One of the tubers was tested by Glynne's method of infection by summer sporangia (10) and found to be immune.

The produce of four other grafted plants of these types was unfortunately lost through disease of various kinds during the 1924 season. In view of the successful building up of grafts of types $5\left(\frac{S}{S}/I\right)$ and $6\left(\frac{I}{I}/S\right)$ and of the results obtained from them, the repetition of the grafts of types 3 and 4 was postponed.

Grafts of type $6\left(\frac{I}{I}/S\right)$. The problem to be solved by grafts of type 6 was whether an immune plant is able to bestow immunity on a susceptible tuber grafted on it and "fed" by it. In each experiment a cleft graft was made uniting the stolon of the grafted-on susceptible tuber to a conveniently sized stolon of the immune plant. It was found advantageous with all stolon grafts to inhibit normal tuber formation of the foster-plant somewhat by uncovering the portion of the stem below soil level and exposing it to light, leaving only the grafted stolon covered. In this way the development of new stolons was avoided. It was found that unless this precaution was taken, storage took place in existing tubers or in those arising on newly formed stolons, so that the grafted-on stolon, though remaining apparently quite healthy, did not increase in size. Normal produce from the foster plant could of course be obtained after the grafted-on portion had grown sufficiently, by merely covering a part of the stalk again. Covering the stalk at this late stage when the graft-union was complete did not prevent further increase in size of the grafted-on portion.

In experiment No. 613 (1) use was made of a very heavily warted piece of Arran Chief (S) tissue. An underground stem of this plant, which had turned upwards, had evidently become infected just as it

[1] I = immune. [2] S = susceptible.

emerged from the soil and when its leaves were just beginning to expand. The whole leafy structure was heavily infected. It was grafted by means of its stem on a K. of K. (I) stolon.

The final condition of this graft is illustrated in Plate XII, fig. 1. The warted tissue can be seen in the bottom left-hand corner of the figure near the sixpence, which gives some idea of the size of the wart. The position of the graft-union is indicated by the binding threads just above the wart. Periodic examination with a lens showed that the actual number of sporangia was doubled during the period of the experiment; hence there was no suggestion of an inhibition of the disease as a result of the warted tissues being "fed" on food material derived from the immune plant. The healthy appearance of the union, the turgidity and the increase in size of the grafted-on part left no doubt that organic union had actually taken place; pieces of warted material similar to that used as the scion, but not grafted, became flaccid in less than a day.

In experiment No. 616 an Arran Chief tuber (S) (diameter about 1 cm.), having on it a wart about 1 mm. in diameter, was grafted by means of its stolon on a stolon of a K. of K. (I) plant. After about a week the wart commenced to grow, though the tuber did not increase appreciably in size. Successive stages in the development of the wart are shown in Plate XIII, figs. 5 and 6[1]. Here again there was no evidence of the immune plant having any inhibiting effect on the development of the wart. Examination of the junction showed that organic union had taken place, for the two vascular systems were joined.

In experiment No. 613 (2) the immune plant, again K. of K., was given an apparently still better opportunity to affect the grafted-on wart, for the latter was of microscopic size, and as far as could be seen from an examination with a binocular microscope, consisted of one summer sorus only. The graft was made on August 11th, 1923[1]. The photograph from which Plate XII, fig. 1 was reproduced was taken on August 16th. Plate XII, fig. 2, is an enlarged view taken on August 17th. Plate XII, figs. 3 and 4, show the condition of the wart on September 4th and 10th respectively. By suitable arrangement of the camera, etc., it was possible to take the last three photographs so that the magnification was the same each time. The diameter of the tuber remained at 0·5 cm.; but, as can be seen from the figures, the wart increased very greatly in size.

[1] Unfortunately no photograph was taken of these tubers at the time when the graft was made.

The behaviour in the above three grafting experiments show that at least so far as the two varieties, Arran Chief (S) and K. of K. (I) are concerned, the immune host plant was unable to inhibit the growth of the wart on the grafted-on tissue, even though that tissue was entirely dependent on the immune plant for all the materials used by it in its growth. There is still the possibility that if the susceptible tissue had been grafted on the immune plant before the wart disease fungus had become established, then the immune plant by means of the products supplied by it to the grafted-on tissue, might have made infection by the parasite impossible. In experiment No. 528 a piece of stolon about 3 cm. long, free from wart disease, raised in uncontaminated soil, and not having yet begun to swell into a tuber, was cut from a Garland (S) plant (white-tubered), and grafted on near the end of a stolon—at that time devoid of tubers—of an Arran Victory (I) plant (purple-tubered). The graft was made on June 3rd, 1923. On July 10th the top soil was carefully washed away, when the grafted-on stolon was found to have increased greatly in length and bore three white tubers of small "seed" size, whilst the foster stolon now bore three purple ones. The clean soil which had been washed away was now replaced by heavily infected material, and in addition, on July 23rd, three warts were placed above the largest white tuber. The whole was kept moist. On August 28th the largest white tuber had two small warts on it. The foster stolon, together with the stolon grafted on it, was cut off close to the stalk, the tubers freed from soil and the whole placed in a glass dish and kept for about a fortnight covered with a piece of damp felt to encourage wart formation. At the end of this time each white tuber had at least one small wart on it, but the purple ones (Arran Victory) were quite free from infection. At this point the tubers were preserved in formaldehyde, as the stolon was beginning to rot. A photograph of the specimen is reproduced in Plate XIII, fig. 7.

During the 1925 season four more grafts of this type were made. In each experiment of this type and of type 5, to be described shortly, the plants were grown in soil free from wart disease until tubers at least as large as hen's eggs had been produced on the grafted-on part, then all the tubers, whether arising directly on the foster plant or on the grafted-on part, were surrounded by heavily contaminated material and treated in such a way as to promote wart formation as far as possible.

No. 901. *A King Edward (S) stolon grafted on an Abundance (I) plant.*

The grafted-on stolon produced one tuber weighing approximately 100 gm. Of the 7 eyes 1 was warted.

No. 905. *A King Edward (S) stolon grafted on a Great Scot (I) plant.*

The grafted-on stolon produced:

Tuber	Weight (gm.)	Eyes warted	Eyes clean
1	88	7	7
2	83	6	3
3	47	2	3
Totals	218	15	13

Four Great Scot tubers produced on the immune foster plant were free from wart.

No. 913. *Up-to-Date (S) stolons grafted on a Kerr's Pink (I) plant.*

Stolon I

Tuber	Weight (gm.)	Eyes warted	Eyes clean
1	125	0	7
2	112	0	14
Totals	237	0	21

Stolon II

Tuber	Weight (gm.)	Eyes warted	Eyes clean
3	53	0	10
4	43	0	6
Totals	96	0	16

One of the "scion" tubers and one of those produced by the foster plant were tested by the method of infection, by summer sporangia, to which reference has already been made(10). The Kerr's Pink tuber remained clean, but the scion tuber became thoroughly infected, showing that the lack of infection on the growing plant was most probably due to the conditions under which the plant was grown not being sufficiently suitable for the production of warts, and not to any effect of the foster plant on the grafted tuber.

The following grafts of type $5\left(\frac{S}{S}/I\right)$ were made during the 1925 season.

No. 912. *An Abundance (I) stolon grafted on a King Edward (S) plant.*

	Produce of grafted-on stolon		
Tuber	Weight (gm.)	Eyes warted	Eyes clean
1	36	0	6
2	20	0	4
3	8	0	2
Totals	64	0	12

Two of these tubers were tested by Glynne's method of infection by summer sporangia (10) and found immune.

	Produce of host plant		
Tuber	Weight (gm.)	Eyes warted	Eyes clean
1	52	3	5
2	43	2	7
3	23	0	8
4	20	0	6
5	10	0	7
Totals	148	5	33

No. 914. *A Kerr's Pink (I) stolon grafted on an Up-to-Date (S) plant.*

The grafted-on stolon produced one tuber weighing 36 gm., all 7 eyes of which were clean, though a tuber borne by a stolon arising on the foster plant, and placed immediately below the other had 3 large eyes infected.

Conclusions.

It may be concluded from the above results, showing as they do that the reaction of any piece of tissue towards wart disease is entirely independent of the influence of any foreign tissue grafted upon it, that the cause of immunity from, or susceptibility to, wart disease is innate in the cell and is unable to move thence except by cell division. Further it is not influenced by substances which are able to pass unchanged across a graft fusion layer and permeate the tissues of the other component of the composite grafted plant.

It is thus evident that susceptibility to wart disease is different in nature from susceptibility to crown gall, for Wormald and Grubb (19) have shown that a scion, susceptible to crown gall, may bestow susceptibility on a resistant stock on which it is grafted.

More definite deductions cannot be drawn until our knowledge of the physiological, and particularly the chemical, effects of stock on scion and scion on stock is increased. By considering the above facts,

however, in relation to those which are known concerning the effects of stock on scion and *vice versa*, guidance may be obtained in choosing which lines of attack on the problem are to be avoided as being unlikely to be fruitful, and which are most likely to yield useful results.

The idea expressed by Köhler(13) that immunity from wart disease is caused by a compound built up in the leaves and transported thence to all parts of the plant, for instance, is almost certainly wrong.

The fact that at least two alkaloids (and also, according to Daniel, solanine(7)) are known to pass across a graft fusion layer, renders unlikely the idea, which has been put forward(12), that solanine is the cause of immunity from wart disease; it seems likely also that the cause of immunity is not explicable in terms of any other compound similar to alkaloids. The same may be said in regard to essential oils and probably tanins (cf. p. 182).

It has already been stated (p. 182) that Barker and Grove have shown that the acidity of apple juice varies according to the stock on which the apple is grafted. It is unlikely therefore that acidity plays any part in rendering varieties immune—a conclusion already reached by Weiss and Harvey(18) as a result of hydrion determinations on potato juice, and by Köhler(12) as a result of allowing potato tissue to absorb an innocuous indicator and thereby determining hydrion concentration of the cell contents.

We have seen that grafting a small piece of stolon on a plant of opposite reaction towards wart disease caused no change in the reaction of this stolon. In experiments Nos. 528, 901, 905, 913, 912 and 914 the stolon, in swelling into one or more tubers, increased in volume at least a hundredfold. This increase in volume may be looked upon as the final result of a series of synthetic processes following on absorption of inorganic salts by the roots, and of carbon dioxide by the leaves, resulting finally in the building up of the more fundamental constituents of the cells. Hence it is only in this last stage that the building up of immune tissue differs from that of susceptible tissue. In seeking the cause of immunity therefore we must, in all probability, look to one or other of those chemical compounds which constitute the more permanent "structures" of the cell. Since the compound carrying the cause of immunity does not appear ever to leave the cell, and its increase is inseparably connected with cell division, it seems likely to be closely associated with those compounds which are the seats of specificity in general; in other words, immunity from wart disease is a property of protoplasm.

About the chemistry of protoplasm little is known, but proteins are invariably constituents of it. The extraordinary specificity of these compounds, as revealed by immuno-chemical, and purely chemical, methods, does not seem to be shared by any other class of compound. What is already known of their chemical structures in no way precludes the possibility of their being the seats of specificity in general. A detailed investigation of the proteins of immune and susceptible varieties of potatoes by immuno-chemical methods seems, therefore, to be the next step in the present problem.

Summary.

It is known that grafting together portions of two different plants is sometimes followed by changes in their chemical contents owing to permeation of each by substances formed in the other.

The present investigation is an attempt to determine by grafting together pieces of immune and susceptible plants whether the cause of immunity from wart disease of potatoes is carried by chemical compounds which can traverse unchanged a graft fusion layer or by those which are unable so to do.

For this purpose all the eight possible types of plants have been built up by grafting together root, shoot and tuber systems from either immune or susceptible plants.

In none of the experiments was the reaction of the tubers towards wart disease seen to be changed.

The deduction is therefore drawn that the cause of the immunity is not carried by any compound which is able to traverse the plant.

In this way the problem is considerably narrowed down, for it is now possible to eliminate many lines of attack as unlikely to be fruitful.

The suggestion is put forward that the examination of the proteins from immune and susceptible varieties by immuno-chemical methods is the most hopeful future line of attack.

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EXPLANATION OF PLATES XII AND XIII.

PLATE XII.

Fig. 1. Exp. No. 613 (1) (bottom left-hand corner, near sixpence). Heavily warted tissue grafted on the stolon of an immune plant. When the graft was made the "wart" was half its final size. The number of wart disease sori doubled after the graft was made (see text, p. 184).

Exp. No. 613 (2) (right centre), photograph taken August 16th.

Fig. 2. Exp. No. 613 (2), photograph taken August 17th.

Fig. 3. Exp. No. 613 (2), photograph taken September 4th.

Fig. 4. Exp. No. 613 (2), photograph taken September 10th.

(Figs. 2, 3 and 4 taken to same scale.)

In Exp. No. 613 (2) a small tuber 0·5 cm. diameter, having on it one single summer sorus, was grafted on the stolon of an immune plant. No photograph was taken when the graft was made. Figs. 2, 3 and 4 show successive stages in the growth of the "wart," though "fed" by an immune plant, which therefore was unable to inhibit the growth of the wart through the organic food supply (see text, p. 185).

PLATE XIII.

Figs. 5 and 6. Two stages (taken to same scale) of Exp. No. 616, showing growth of wart on a small potato after being grafted on a stolon of an immune plant. No photograph taken when graft was made (see text, p. 185).

Fig. 7. Exp. 528. Graft union at *A*, cut from stalk of plant at *B*.

A piece of white stolon (below *A*) cut from a susceptible plant was grafted at *A* on the purple stolon (above *A*) of an immune plant. The tubers were produced in soil free from wart disease. Finally when the tubers had arrived at practically their final size they were surrounded with contaminated material. All three tubers on the grafted-on stolon became infected (warts may be seen at *C* and *D*), whereas the ones on the foster stolon remained quite free from wart disease (see text, p. 186).

(*Received November 25th*, 1926.)

Fig. 2.

Fig. 3.

Fig. 1.

Fig. 4.

ROACH.—Immunity of Potato Varieties from Attack by Wart Disease Fungus (pp. 181—192).

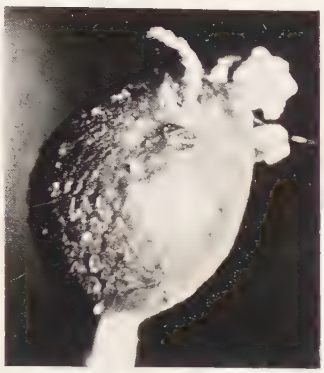

Fig. 5.

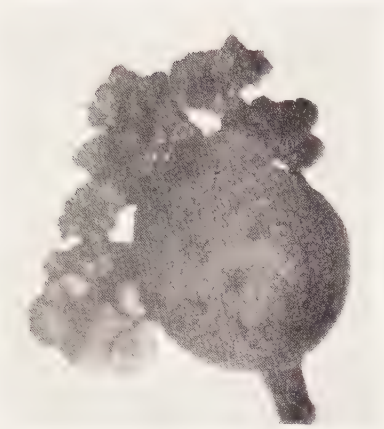

Fig. 6.

Fig. 7.

ROACH.—Immunity of Potato Varieties from Attack by Wart Disease Fungus (pp. 181—192).

AN OAK LEAF DISEASE CAUSED BY *SCLEROTINIA CANDOLLEANA* (LÉV.) FUCKEL.

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(With Plate XIV.)

During the last two years the authors have had under observation a disease which has caused extensive damage on the oak in various parts of Britain, especially in the north. The disease leads to the formation of conspicuous brown blotches on the leaves; in some localities almost the whole of the foliage is discoloured and, in consequence, the impression is given that the tree has been killed. The first specimens were obtained from Perthshire and Aberdeenshire in the summer of 1925 and subsequently in the same year several inquiries regarding the disease were received from widely separated areas in Scotland. In 1926 the disease reached epidemic intensity over the whole of Scotland south of Inverness, and was observed in the greater part of England and also in Wales. Both *Quercus pedunculata* and *Q. sessiliflora* are attacked, and the disease has also been found on nursery plants of *Q. rubra*. It has been recorded by several authors on *Castanea sativa* as well as on *Quercus Robur*.

The disease is caused by *Sclerotinia Candolleana* (Lév.) Fuckel. This species has been known in Britain since 1878 (1) but has been usually regarded as a saprophyte. Massee in 1910 suggested that living leaves might be infected but it appears that no infection experiments have been previously carried out.

On examination only yellowish hyphae can be seen in the discoloured tissue, during the summer, but after the leaves have fallen, small black sclerotia soon appear and, in consequence, this stage of the fungus was previously known as *Sclerotium Pustula* D.C. and *S. quercinum* Pers.

In the past the fungus has been placed in various genera and the following names are synonymous: *Peziza De Candolleana* Lév., *Phialea Candolleana* Quel., *Hymenocypha Candolleana* Phill.

Short accounts of the disease have been previously published in the *Transactions of the Royal Scottish Arboricultural Society* (5) and in the *Gardiner's Chronicle* (6).

The yellow spots on the oak leaves have been first observed towards the end of June. In the early stages they are small and roughly circular (Fig. 1) but gradually increase in size until almost the whole of the leaf may become discoloured. The infected tissue towards the centre of the spot is light brown, and this is surrounded by a darker brown zone. As the season advances the tissue within this dark zone often falls away, leaving a hole in the leaf (Fig. 2). The mycelium in the infected tissue is made up of light yellowish hyphae which ramify in all directions through the cells of the leaf. Secondary fungi are often found on the dead areas, and a species of *Cladosporium* frequently forms a dark dot in the centre of the discoloured tissue. The presence of this fungus was referred to in a previous note (5), where it was suggested that this might possibly represent a conidial stage in the life history of *Sclerotinia Candolleana*. The incorrectness of this suggestion has since been demonstrated by culture and infection experiments.

Sclerotia have not been observed on the leaves while still on the tree, but these soon develop on the fallen leaves (Fig. 2), especially under damp conditions, and they may be also readily obtained by keeping diseased leaves in a moist atmosphere in the laboratory. The sclerotia begin to develop in the leaf tissue and, as they increase in size, burst through the epidermis so that, when mature, they lie freely exposed. A sclerotium usually extends through the thickness of the leaf, but generally develops more strongly towards either the upper or the lower surface. The greater development nearly always takes place towards that side which happens to be uppermost when the leaf is lying on the ground. This ensures that when germination of the sclerotium takes place the fructification is produced freely on the exposed and illuminated surface (Fig. 3). The sclerotia are pulvinate, black, about 0·4 cm. in diameter.

Sclerotia were produced freely in October from a fortnight to three weeks after the leaves were brought into the laboratory. After their formation the leaves with the attached sclerotia were removed from the laboratory and kept outside fully exposed to the weather and, during the winter, were frozen several times. Attempts were made to germinate these sclerotia at various times throughout the winter and spring, but these resulted in failure until the end of May. Germination then took place rapidly, the mature apothecia were produced in about a week

(Fig. 3). Only one apothecium grew out from each sclerotium (Fig. 4). Massee (3) describes the development of 3–4 ascophores from the sclerotium but this was never observed. The stalk of the apothecium is slender, yellow in colour, paler towards the base and varies considerably in length, depending on the amount of illumination and the depth of the debris under which the sclerotium is buried. The stalk attains such a length that the apothecium is borne well above the substratum. Diffuse light tends to lengthen the stalk and, when grown under lateral illumination, the growth is always towards the strongest light. The stalks shown in Fig. 3 were up to 4 cm. in length, but these were developed in weak light, while others in full illumination only reached 0·6–1·2 cm.

The apothecia are at first closed but soon open and become flat when mature and are then about 0·4 cm. in diameter. They are reddish to yellowish brown in colour and are usually rather darker on the upper surface. The structure of the hymenium is quite typical of *Sclerotinia* (Fig. 5). The ascospores are elliptical 7–9 × 3–4 μ, and the paraphyses are slender and slightly thickened towards the apex (Fig. 6).

An account of the germination of the sclerotia has been given by Rostrup (4, 2). He found that in Denmark germination took place in February and March in the laboratory, but in the forest was delayed until June.

In order to test the parasitism of the fungus, mature apothecia still attached to sclerotia were placed so that the ascospores were naturally shot out upon leaves borne on young plants of *Quercus pedunculata*. As a result brown areas appeared on the leaf in 10–14 days and these on examination were found to contain the hyphae of the fungus.

Cultures on artificial media have been made in the autumn from portions of the diseased leaf, and in the spring from the ascospores. The results in each case are quite similar. A white rather fluffy mycelium is produced; no conidia are formed but sclerotia develop within about a month. These sclerotia are often larger than those found on the leaf and are irregular in form. In plate cultures the sclerotia are not produced in any definite position, but are usually formed at the edge of the medium.

The disease is confined to the leaf and does not spread to the shoots and, in consequence, after the leaves have fallen, the trees are no longer infected. This being the case the collection and burning of the fallen leaves, if thoroughly carried out, should entirely remove the source of infection and hence prevent the disease in the following year.

It appears that wherever the fungus is present the majority of the

leaves become infected and in consequence the disease is very obvious and unsightly. As, however, only leaves are infected, it is improbable that trees will be killed unless the defoliation is very marked and continues over a number of seasons.

Two cases of slight infection in forest nurseries have been observed, on *Quercus pedunculata* and *Q. rubra* respectively. These were both in the vicinity of mature trees which were bearing infected leaves. From a consideration of the life history of the fungus it is obvious that severe infection can only take place within a limited distance of infective material, and it is improbable that the disease will prove to be serious in nurseries, unless these are in proximity to mature trees.

As already pointed out, *Sclerotinia Candolleana* has been recorded fairly frequently in the past, both in England and Scotland, but it does not appear to have occurred in great abundance. We are unable to account for its appearance as an epidemic during the last two years. It is very improbable that there has been any change in the distribution of either the fungus or its host plant, and we can only suggest that some climatic condition has favoured its rapid increase.

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EXPLANATION OF PLATE XIV.

All figures refer to *Sclerotinia Candolleana* (Lév.) Fuckel. on *Quercus pedunculata.*

Fig. 1. Leaf showing brown spots in early stage of the disease.

Fig. 2. Leaf bearing sclerotia.

Fig. 3. Leaf showing sclerotia bearing apothecia. × about 2.

Fig. 4. A single sclerotium bearing an apothecium. × about 3.

Fig. 5. Longitudinal section through apothecium. × about 70.

Fig. 6. Part of longitudinal section through apothecium, showing asci and paraphyses. × about 200.

(*Received November 25th*, 1926.)

Fig. 1.

Fig. 3.

Fig. 5.

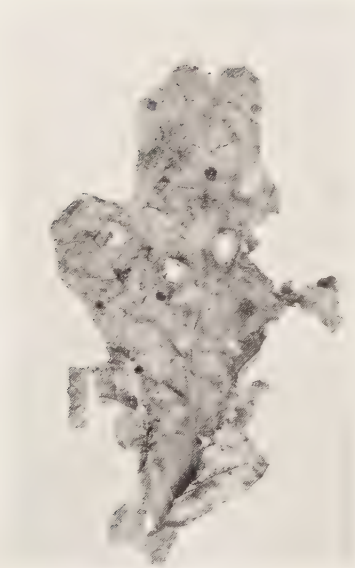

Fig. 2.

Fig. 4.

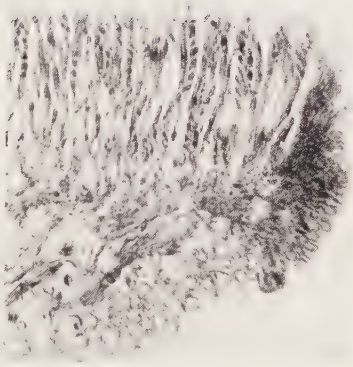

Fig. 6.

WILSON & WALDIE.—An Oak Leaf Disease (pp. 193—196).

THE STRAWBERRY DISEASE IN LANARKSHIRE

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During the spring of this year the author's attention was directed to the strawberry disease in Lanarkshire. Field observations were made throughout the season and a considerable amount of experimental work has also been carried out. The results are summarised in the following preliminary report.

At the outset the author wishes to point out that the "Lanarkshire Strawberry Disease" is not necessarily the same as the disease in England. "John Ruskin," a variety cultivated over a large acreage, has been most affected.

In the field two aspects of the disease are apparent, namely, the devastation *en masse* of square yards of strawberry beds, and the obvious diseased condition of individual plants. In estimating the probable cause of the disease it is important that both of these aspects should be borne in mind, since it is clear that the organism or organisms causing the disease not only are capable of quickly destroying individual plants but also are capable of spreading rapidly through the strawberry beds.

It is not intended here to enter into full details concerning the symptoms of the disease, but a short reference to the soil, the strawberry plants, and possible parasites will make the general finding clear. The soil in the infected fields is for the most part heavy and close, sometimes almost a clay, and seldom sufficiently free for healthy root development. It is acid, though not to an excessive degree. Further it lacks humus and has not the proper coherence and openness. Where the disease is worst the drainage is bad; the soil is waterlogged in wet weather and practically hard-pan in dry. As a whole the soil cannot be regarded as being in a first-rate condition.

Such soil conditions necessarily tell against the vigorous healthy growth of the strawberry plants. For the most part in the areas where the disease is prevalent, it cannot be said that they are growing under the most favourable conditions. When lifted the roots of many of the plants, though not actually diseased, are in a weak and lax condition,

consequent on the excessive stiffness of the soil, combined with lack of adequate ventilation. In short the field observations all tend to indicate that the state of the plant itself, and not the causative organism of the disease, is of first importance. It would thus appear that the poor root system with its soft and limp roots, and the starved condition of the plant, are due to unfavourable soil conditions plus fungal competition. These general conditions which tell against the plant favour the fungal flora of the soil, and thus the plants are rendered very susceptible to fungal and other diseases.

In general the diseased plants are of squat stature, with discoloured leaves (often with red margins); they fail to form fruit, and there is a decline in the formation of runners, all of which facts suggest starvation conditions. Examination of the root system shows that the roots may be completely or partially rotted. This is true of any kind of root. In the case of very young roots and rootlets the disease may first appear as blemishes or discolorations, which in a more advanced condition resemble the typical "damping-off" effect of seedlings familiar to growers. In stouter roots there is a somewhat similar state as the dying back takes place. Sections through a diseased root show the presence of fungal filaments which completely exhaust the storage materials of the cortical cells. A section taken above the diseased region shows cortical cells of normal appearance, free from hyphae, and filled with starch grains. In plants where the disease was quite far advanced it was found that the penetrating fungal hyphae were confined to the roots, and that the root stock, petioles and leaves (although showing the results of the disease) were free from fungal hyphae. Young blemished roots and rootlets were found to have hyphae associated with them.

Without prejudging the evidence in any way the observer is struck by the absence in a diseased plant of those fine roots and rootlets which play the chief part in the actual absorption of the mineral salts and water from the soil. It is concluded that the destruction wrought on the fine absorbing roots is responsible for the various signs of starvation perceptible in the green aerial part of the plant and for its final decline and death, in fact for all the symptoms usually associated with "Lanarkshire Strawberry Disease."

Organisms causing the Disease.

Wounding organisms of various kinds, such as eel worm, cannot at this stage be ruled out altogether as first causes in the attack; the general evidence, however, points to the fact that the ultimate and rapid

decline in the vitality of the strawberry plant is due to the exploitation of the root system (especially of the fine absorbing roots) by fungal hyphae.

Using the usual mycological technique, attempts were made to isolate the fungus or fungi concerned in the decay. For this purpose roots of various kinds were selected, viz. fine roots 2 cm. long, arising from the base of the stem, old roots showing blemishes or badly attacked, and rootlets arising from these. Where possible the fungi were extracted from the junction of decayed and undecayed tissue after superficial sterilisation with solutions of mercuric chloride or formaldehyde. Eighteen separate extractions of fungi were made in this manner, and on twelve occasions *Pythium* sp. was the only fungus obtained. On the other six occasions the fungi isolated belonged to the Fungi Imperfecti. The *Pythium* cultures were obtained from several batches of plants and extracted at different times. *Pythium* was found associated with both young and old blemished and decayed roots.

Inoculation tests were then carried out. This was done by allowing young strawberry plants (from runners) to strike in pots of sterilised soil, care being taken to avoid contamination from the various external sources. When the plants were well rooted the pots were inoculated with the cultures of *Pythium* which had been extracted. After fourteen to twenty-one days the plants became diseased and typical symptoms were developed. It was found that the *Pythium* was capable of attacking both young and old roots, causing a die-back of the thick roots, and denuding them of their fine fibrous rootlets. Under the same conditions non-inoculated control pots remained healthy. The affected roots in the inoculated pots were taken into the laboratory and the *Pythium* was again isolated from them.

This evidence demonstrates that the *Pythium* is capable of producing the strawberry disease as it occurs in field conditions. These observations however do not necessarily rule out other causes of disease. Some inoculation experiments were also carried out with another of the isolated fungi, and this also proved capable of attacking the strawberry roots. Under conditions unfavourable to the plant it is probable that a number of the soil fungi will prove capable of attacking the roots. It is also probable that under special conditions a great many of the soil saprophytes may develop parasitic tendencies. This tendency will be furthered by any reduced vigour of the crop plants. Further experiments along these lines are in progress. A full account of the isolation of the fungi and methods of inoculation will be set forth in a later publication. In

the field it has been found that there are two periods of fungal attack, namely, spring and autumn. The isolations referred to above were all made from the spring infection. It is possible that isolations from the autumn infection will reveal some additional parasites.

Without stressing such evidence as there is to hand at the present time it appears to the author that whether *Pythium* is or is not the primary agent causing the disease, there can be no doubt that it has the power of exploiting the root system on which the general health of the whole plant depends. Further, it is to be noted that *Pythium* sp. is a fungus which exploits quickly and spreads very rapidly, both of which features are in accordance with the striking aspects of the disease as seen in the field.

In the light of these observations it is clear that the problem is probably an agricultural rather than a mycological one. The main factor involved appears to be the general health of the plant. The fungal attack is to be regarded as an important accessory to the main factor. It is, however, a factor which has become increasingly aggressive owing to the accentuation of certain soil conditions. If the roots reach a sufficient state of laxness and ill-health they are liable to be attacked by any of the parasitic soil fungi. Thus the disease may be due to fungal agency without necessarily being referable to one parasite only in all cases.

In view of this finding, experimental work with the soil is being carried out side by side with the mycological studies.

Summary.

1. In the field two aspects of the "Lanarkshire Strawberry Disease" are apparent, namely, the devastation *en masse* of the strawberry beds, and the diseased condition of individual plants.

2. Soil conditions as a whole are unfavourable, and this tells against the development of an extensive and healthy root system.

3. The roots are weak and lax, consequent on the excessive stiffness of the soil. This renders them liable to fungal attack.

4. Roots of all kinds were found to be penetrated by fungal hyphae, and to have hyphae associated with them.

5. Eighteen separate extractions of fungi were made, and on twelve occasions a species of *Pythium* was the only fungus obtained.

6. When inoculated into pots in which strawberry plants had been grown under sterile conditions, the *Pythium* attacked young and old roots, and produced all the symptoms of the disease.

7. The *Pythium* was isolated again in the laboratory from inoculated plants.

8. This evidence demonstrates that the *Pythium* is capable of producing the strawberry disease as it occurs in field conditions. It is concluded that *Pythium* is an important factor in exploiting the root system on which the general health of the plant depends.

9. The problem appears to be an agricultural rather than a mycological one. The main factor involved appears to be the general health of the plant. The fungal attack is to be regarded as an important accessory to the main factor. It is, however, a factor which has become increasingly aggressive owing to the accentuation of certain soil conditions.

10. Experimental work on the soil is being carried out side by side with the mycological studies.

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ANTAGONISM OF MICRO-ORGANISMS AS THE CONTROLLING FACTOR IN THE INHIBITION OF SCAB BY GREEN-MANURING

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(With Plates XV–XVII.)

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Introduction.

Millard (4) has shown that common scab of potatoes may be inhibited by thoroughly mixing a quantity of green organic matter with the soil before planting. To account for this he put forward the hypothesis that since *Actinomyces scabies* can live saprophytically it will continue to do so as long as there remains a sufficiency of suitable organic residues in the soil, and that in this way a protective action is exerted by the green manure on the potato tubers. There is here a tacit assumption that the fungus behaves in a similar manner in nature as in artificial media where its habit is to form a compact lichenoid-like growth on the substratum rather than to spread in the manner of most fungi. For some time it has been felt that some other reason might be found to underlie the phenomenon. It is well known that where, in artificial culture, two micro-organisms are grown on the same plate culture or on the same slope, the one may become dominant even to the total extinction of the

other. The dominant organism is naturally that which is the better adapted for growth on the media employed. A like competition takes place among micro-organisms in the soil, and it occurred to us that therein might be found a solution of the problem. "Scabbing soils" are naturally low in organic content, and, as an example, we may mention one of the worst "scabbing soils" with which we are acquainted where the loss on ignition was only 3·8 per cent. Organic matter whether of animal or vegetable origin quickly disappears in such soils and its decomposition is accompanied by great increases in the micro-flora. Conn (1) cites an increase in *Actinomyces* content of 10,000,000 per gram when grass roots were added to the soil. Millard and Whittle in some unpublished work have found that the initial increase of the *Actinomyces* after green manuring is extremely rapid in light soils. Naturally those species which will most readily respond to this growth impetus will be the obligate saprophytes. There seems little doubt, however, that parasitic species are also able to live on organic remains in the soil, since, as shown by Jones and Edson (2), B. F. Lutman (3), and Millard and Burr (6), scab may appear on potato crops grown in soils which have either never grown a root crop or have been out of cultivation for 50 years or more. Moreover, Millard and Burr (7) have recently shown that there is a wide variation in the degree of parasitism shown by certain members of the group, and it may well be that the saprophytic capacity of any species is in inverse ratio to its parasitic potentiality.

Thus, the addition of green manure to a light soil may be expected to lead, at first, to an increased growth of both saprophytic and parasitic *Actinomyces* types. Again, where the vegetable matter added is in a sufficient state of fineness and brought into a close admixture with the soil, it is reasonable to suppose that a very large proportion of the *Actinomyces* population will eventually become congregated upon it. Here, then, conditions become comparable to those in a mixed artificial culture. There can be little doubt but that the obligate saprophytes will become dominant, and, that being so, it is very possible that either by their secretion of toxic substances or by reason of their greater success in the competition for the available food supply they will depress or even kill off the more weakly growing parasitic species. Such an hypothesis easily lends itself to experimental proof, and the experiments to be described were designed for that purpose.

Plan of Experiments.

Pots of sterile soil were inoculated in varying proportions with cultures of two species of *Actinomyces*—one a virulent parasite and the other an obligate saprophyte. In the first experiment, the inoculations were made in such a way that in the series there was a gradually increasing amount of the saprophyte and a correspondingly decreasing amount of the parasite, whilst, in the second and third experiments, the inoculum of the parasite given to each pot was constant, and that of the saprophyte only was made to vary. Other series of pots were set up in each experiment, in which grass cuttings were mixed with the soil before sterilising, and these were similarly inoculated. Potatoes were then grown in the pots.

During the growth of the plants and immediately after lifting, soil samples were taken from the pots and counts made of the two *Actinomyces* species. Unfortunately, no method has so far been evolved whereby any absolute number representing the *Actinomyces* content of the soil can be obtained, but, by following a conventional technique, the counts may be regarded as giving correct relative values—not as between species and species but as between periodical counts of the same species from the different pots. From them it was possible to ascertain the extent of the competition between the organisms, the influence of the grass in this connection and the correlation, if any, between these effects and the scabbing of the respective crops.

Experiment 1.

The soil was a light loam having no lime requirement. Three series (*A*, *B* and *C*), each containing 4 pots, were prepared as follows:

Series *A*. Soil only.

Series *B*. Soil and grass cuttings intimately mixed—the amount of grass used being such as would inhibit scab under ordinary field conditions.

Series *C*. Soil and grass cuttings, the latter in twice the quantity used in *B*.

All the pots, when filled, were sterilised at 3½ atmospheres pressure for 20 minutes and then placed in a greenhouse which had been thoroughly washed and disinfected. For the inoculations the two species of *Actinomyces*, *A. scabies* (Thaxter) Güssow emend. M. and B. and *A. praecox*, from Millard and Burr's (8) stock cultures were selected. The former of

these is a virulent parasite and the latter an obligate saprophyte. Cultures of both species were made on potato plugs, and the pots were inoculated by shaking the cultures into small holes made in the soil. They were then cut into small pieces with a sterile knife and thoroughly mixed in to a depth of 6 inches. The relative proportions of inoculum given in each series were as follows:

Each series. No. of pot	No. of cultures used	
	A. scabies	*A. praecox*
1	12	0
2	8*	4
3	4*	8
4	0	12

* It was known from previous experiments that an inoculum of 6 potato plug cultures of this organism would produce a scabbed crop.

After two weeks each pot was planted with a seed potato (variety Field Marshal), which had been sterilised by soaking in a $\frac{1}{6}$ per cent. solution of formaldehyde for two hours and subsequently allowed to sprout in a sterile box. Later, each pot was given a teaspoonful of a previously sterilised mixture of artificial manures. The benches and the floor in the greenhouse were washed each week with an antiseptic solution and the house was twice fumigated with tetrachlorethane during the experiment. The plants were watered with sterilised tap-

Table I.

Exp. 1. *Degree of scab on crops.*

Series	No. of pot	Description of crop	Virulence
A	1	21 small potatoes all badly scabbed and showing deep brown fissures which, in some cases, covered the whole of the potato	10
	2	7 potatoes of which 5 were scabby	5
	3	10 potatoes, 2 only showing scabs	3
	4	All tubers clean	0
B	1	19 potatoes, 14 badly scabbed showing fissures as in Series *A*, Pot 1	9
	2	15 potatoes, 8 showing small scabs	3
	3	10 potatoes, 1 only showing scab	1
	4	14 potatoes, all clean	0
C	1	11 potatoes, unfortunately badly attacked by *Rhizoctonia solani**, a few small scabs present	1
	2	7 potatoes, 2 scabby	2
	3	8 potatoes, all clean	0
	4	4 potatoes, all clean	0

* Tubers severely attacked by this fungus are rarely badly scabbed.

water and in every case produced good crops. One sample of soil was taken from each pot during the growth of the plants and one after the potatoes had been lifted for counts of the *Actinomyces* present.

Results of Experiment 1. The crops were lifted 20 weeks after planting, and the amount of scab present in each is shown in Table I. The virulence of the disease is indicated by numbers with a maximum of 10.

It is unfortunately not possible to reproduce photographs of all the crops in the different series. Those from Series *B* have been selected and are shown in Fig. 1, where each row of three tubers is representative of the whole crop. The crops from Series *A* were so similar that each might be taken to be a duplicate of that from the corresponding pot in Series *B*. In Series *C*, however, the crops from Pots 2, 3 and 4 were cleaner than the corresponding crops from Series *A* and *B*. The following points appear from the experiment:

(1) That the green manuring in Pot 1, of Series *B*, has not appreciably reduced scab.

(2) That a reduction in scab has taken place in each pot whether of Series *A*, *B* or *C* where inoculations of the saprophytic species have been made. This decrease of scab is so great that in Pot 3 of each series the tubers were practically clean in spite of the inoculations of the parasitic species. In regard to Series *B* and *C*, where green manure had been applied, this result was to be expected, but it was at first very surprising to find that scab had also been inhibited in Series *A* where soil only was used. We attribute this, however, to the fact that a heat sterilised soil is in itself a medium in which rapid Actinomycetal growth will occur and the conditions are thus comparable to that in which an additional food supply has been added.

Soil Counts. The two species of *Actinomyces* used in the inoculations behave very differently in culture and no difficulty was apprehended in distinguishing between them on nutrient media. On nutrient potato agar *A. scabies* produces an umbonate fleshy colony which forms no aerial mycelium but stains the medium a deep brown colour. *A. praecox*, on the other hand, produces a white aerial mycelium and does not stain the medium. In practice, however, great difficulty was at first experienced. It should be remembered that during the period of growth of the potatoes, a re-population of the soil with bacteria and fungi was bound to occur. When soil samples are plated out, the bacterial colonies are the first to appear and these may be so numerous as to prevent the subsequent growth of the *Actinomyces*. Even in the plates of the higher dilutions, bacterial colonies exert sufficient influence to change the char-

acters of the *Actinomyces* colonies. Thus, on nutrient potato agar it was found that colonies of *A. scabies* frequently failed to produce any stain, whilst colonies of *A. praecox* sometimes formed no aerial mycelium. Such colonies could therefore only be identified by isolating and growing them in pure culture—a long and tedious process. Again, it was difficult to distinguish between the species when the colonies grew below the surface of the medium. Thornton's synthetic agar and soil extract agar were substituted for nutrient potato agar but were not found to give any more satisfactory results and the counts made were ultimately discarded as valueless. Subsequently, egg albumen agar was found to be a very suitable medium and was employed with success in the later experiments. Here, however, for reasons given later, no appreciable difficulty was caused by bacterial contamination and even nutrient potato agar could be used without trouble.

Experiment 2.

The procedure followed in this experiment differed from that of Exp. 1 in the following particulars:

(1) The soil was of an extremely light and porous nature in which *Actinomyces* were known to thrive particularly well.

(2) Two series, each consisting of 10 pots (in 5 duplicate pairs) were prepared, Series *A*, of soil only, and Series *B*, of soil mixed with grass cuttings. As this experiment was started in the early spring it was impossible at the time to obtain lawn cuttings, and it did not occur to us that under greenhouse conditions Timothy grass grown in the pots and forked in would have answered the same purpose. However, the inoculations were made in both series of the sterilised soil, and the grass cuttings which became available on March 1st, were then added (after sterilisation) to the pots in Series *B*.

(3) In the inoculations each pot, excepting Nos. 9 and 10, was given

Each series. No. of pot	No. of cultures used as inoculum		
	A. scabies	*A. praecox*	
1	24	0	duplicate pots
2	24	0	
3	24	6	
4	24	6	" "
5	24	12	
6	24	12	" "
7	24	24	
8	24	24	" "
9	0	24	
10	0	24	" "

the same amount of inoculum of the parasitic species, whilst the inoculum of the saprophytic species was increased in geometrical progression in each series. The number of cultures given to each pot is shown in the table on p. 207.

Two additional pots which received no inoculum of either species were included to test the sterility of the soil. It will be noticed that the inoculum of 24 plug cultures of the parasitic species was an extremely heavy one and constituted a very severe test for the theory, even where the saprophyte was added in correspondingly heavy amounts.

(4) Two varieties of potatoes, Epicure and Sharp's Express, were used and one of each was planted in the duplicate pots on March 5th.

In all other respects the technique of the experiment was similar to that of Exp. 1. One soil sample was taken from each pot on April 11th, 5 weeks after inoculation of the soil, and another immediately after the potatoes were lifted on August 11th.

Results of Experiment 2. These are best appreciated by reference to Fig. 2 where again each row of tubers is representative of the respective crop or crops from duplicate pots of Series *B*. Photographs of four crops only are given since these sufficiently represent the gradation in degree of scab found in the series. The following observations may be made:

(1) In Pots 1 and 2 of each series every tuber was literally a mass of scab (Fig. 2, row 1). As in Exp. 1, green manuring in the absence of a saprophytic *Actinomyces* species has not reduced the degree of scab in the slightest degree.

(2) A gradual reduction in the amount of scab occurred along each series but, in contradistinction to Exp. 1, this was a little more marked in Series *B* (green manured) than in Series *A* (soil only).

(3) In Pots 7 and 8 (Fig. 2, row 3), where the maximum inoculum of the saprophyte was given, scab has not been entirely inhibited. The reduction from that of Pots 1 and 2 was, however, very great and quite comparable to what one might expect under natural conditions in a green manuring experiment on a soil so heavily loaded with the parasite.

Soil Counts. The first counts were made on April 11th, 1926. Possibly on account of the enormous *Actinomyces* flora introduced into the pots, the bacterial flora was found to be greatly reduced and bacterial colonies on the plates caused practically no trouble. Both egg albumen agar and nutrient potato agar were therefore used for the platings. On the former medium *A. scabies* produces a large spreading colony, yellow on the underside, and forming a central tuft of white aerial mycelium, whilst *A. praecox* produces a thin flesh-coloured colony on which aerial

mycelium quickly appears, rimming the colony and then forming one or more concentric circles within the first. Where any doubt occurred as to the identity of any colonies, *e.g.* submerged colonies, these were picked off from the millionth dilution plates and grown on nutrient potato agar slopes. Each soil sample was plated in quadruplicate and, excepting in the cases of Pots 2 and 5, where the plates were spoiled by "spreaders," the four series of plates agreed very closely. A fair correspondence was also found between the duplicate pots, *i.e.* Nos. 1 and 2, 3 and 4, and so on, excepting in Pots 7 and 8, Series *A*, and Pots 3 and 4, Series *B*. The figures given in Table II are the averages of the counts from the quadruplicate platings.

Table II.

Exp. 2. *Count of* Actinomyces *from soil samples taken April* 11*th*, 1926.

No. of pot.	Average No. of *Actinomyces* colonies per gram of air-dried soil counted from the millionth dilution plates after 20 days at 24° C.	
Series *A*	*A. scabies* (millions)	*A. praecox* (millions)
1	1·9	0
2	Plates spoiled	—
3	0	52·0
4	0	98·0
5	Plates spoiled	—
6	0	102·5
7	0	14·0
8	0	174·0
9	0	227·0
10	0	234·0
Series *B*		
1	8·5	—
2	9·5	—
3	0	12·5
4	0	110·0
5	0	169·0
6	0	183·0
7	0	394·0
8	0	124·0
9	0	122·0
10	0	135·0

From Table II the following observations may be made:

(1) The population of *A. praecox* in all pots inoculated with this species is extraordinarily great but there is no indication that this has been appreciably increased by the addition of grass in Series *B*. In this

connection, however, as already pointed out in Exp. 1, due allowance must be made for the fact that we are dealing with sterilised soil in both series and this serves to mask the effect of the grass in Series *B*.

(2) *A. scabies* has shown an increase in the green manured Pots 1 and 2 of Series *B*, over that of the pots containing soil alone in Pots 1 and 2 of Series *A*. Thus, in the absence of saprophytic species, the addition of grass may even lead to an increase of the scab-producing organism.

(3) The table indicates an entire absence of *A. scabies* from every pot which received inoculation with *A. praecox*. This statement, however, needs qualifying since in the greater number of platings counts were only possible from the millionth dilution plates. The figure of the count is most accurately interpreted as meaning absence of *A. scabies* colonies in the millions degree.

A second count using egg albumen agar was made on August 11th, 1926, immediately after lifting the potatoes but was limited to the pots of Series *B*. The results are given in Table III.

Table III.

Exp. 2. *Count of* Actinomyces *from soil samples taken August 11th*, 1926.

No. of pot. Series *B*	Average No. of *Actinomyces* colonies per gram of air-dried soil counted from the millionth dilution plates after 20 days at 24° C.	
	A. scabies (millions)	*A. praecox* (millions)
1	34·5	0
2	79·5	0
3	2·5	123·0
4	4·0	128·5
5	9·0	148·0
6	4·5	123·0
7	2·5	130·0
8	3·0	141·5
9	0	172·0
10	0	206·0

Here it is obvious that the parasite, though considerably depressed, is by no means killed off as might have been inferred from the first count. The difference between the two counts in respect of *A. scabies* is probably due to the sampling. Thus, the samples for the first count were taken whilst the potatoes were growing in the pots from small holes 3 to 4 inches deep made towards the edge of the pot. The second samples were taken after the soil had been again thoroughly mixed. It seems probable then that *A. scabies* thrives more readily in the lower

and damper soil layers. The results given in Table III are certainly more in accordance with the degree of scab found on the crops.

It was thought very possible that the inhibitory effect of *A. praecox* on *A. scabies* might not have reached its maximum at the time of lifting the crop and that a second crop grown in the same pots might show yet further evidence of this repression. A further experiment was therefore started.

Experiment 3.

Without any renewal of the soil or further inoculation each pot of Series *B* was replanted on August 20th with a sterilised tuber of the variety Great Scot. The crops were lifted on November 15th and, although the tubers were rather small, the results were of great interest. As regards scab these were as follows:

Pots 1 and 2. Tubers a mass of scab as in the previous crop.

Pot 3. Scab slight.

Pot 4. Scab very severe.

Pots 5 and 6. Scab slight.

Pots 7 and 8. Tubers almost free from scab.

Pots 9 and 10. Tubers quite clean.

Photographs of the crops from Pots 4, 6, 8 and 10 are shown in Fig. 3. In Pot 3 the skin of the tubers was very rough and hard and it seems as though here infection of the tubers had been modified by some secondary cause. With the exception of this pot the series showed a progressive diminution of scab, which was much more pronounced than that of Exp. 2 and entirely in accord with what had been expected.

Soil samples were taken after lifting the crops from Pots 2, 4, 6 and 8, and later, on account of the apparent anachronism shown by the count of Pot 4, a sample was also taken from its duplicate Pot 3. The counts were made on nutrient potato agar and are given in Table IV.

Table IV.

Exp. 3. *Count of* Actinomyces *from soil samples taken November 18th*, 1926.

No. of pot	Average No. of *Actinomyces* colonies per gram of air-dried soil counted from the millionth dilution plates after 20 days at 24° C.	
	A. scabies (millions)	*A. praecox* (millions)
2	225	0
3	—	119
4	7	202
6	—	142
8	—	180

This count reveals two points of interest. The first is the extraordinary further increase in the *A. scabies* content of Pot 2 as compared with the two previous counts of the same soil in Exp. 2. From the lower temperatures of the greenhouse during the two months previous to lifting we might have expected a fall rather than a rise in the *Actinomyces* content of the soil, since it is well known that the parasitic members of the group are most active at relatively high temperatures. Again, such evidence as is available points to the fact that low temperatures are not conducive to spore production. It seems only possible then to account for this increased content of *A. scabies* by the long-continued growth in the soil of its natural host. Millard and Burr (9) have shown that this particular species of *Actinomyces* attacks the roots as well as the tubers of the potato and thus the growth of two successive potato crops will have provided a plentiful and well-distributed supply of suitable food to the parasite throughout the year.

The second point of interest is the absence of the parasitic species from the millionth dilution plates of samples from Pots 3, 6 and 8. This result confirms the previous counts and fully establishes the depressing effect of the saprophyte on the parasitic species. Pot 4 furnishes an exception which can only be accounted for by the fact that (along with Pot 3) it received the smallest inoculum of the saprophyte. For some reason too, the soil here was very much damper when the crops were lifted than that of the other pots, and this condition may have favoured the parasitic species. The close correspondence between this count and the degree of scab produced in the pots is well illustrated by the photographs (Fig. 3).

Discussion.

It has been shown that in a soil previously sterilised a saprophytic species of *Actinomyces* will depress the growth of a virulent scab producing species to the point at which its parasitic attack is negligible. Whilst, under the conditions of the experiments this inhibitory effect has shown itself in sterilised soil nearly as strongly as in a mixture of sterilised soil and grass, reasons have been advanced for this and there is little doubt that in the field the effect would be very dependent on green manuring. We may now ask wherein lies the incompatibility between these two organisms. The question of soil reaction first suggests itself. Millard (5) in his work on the control of scab, found that the addition of green vegetable matter to the soil brought about a slight decrease rather than an increase in acidity, and, more recently, Sanford (10) records

a similar result. It was thought desirable, however, to ascertain what change in soil reaction, if any, had taken place in our experiments. On the conclusion of Exp. 3, therefore, estimations of the *p*H values of the soil in each of the pots in Series *B* were made, using Gillespie's method, and the results are given below.

*p*H *values. Estimations made three weeks after lifting the potato crops in Exp.* 3.

No. of pot	*p*H	
1	7·3	duplicate pots
2	7·7	
3	7·0	" "
4	6·9	
5	7·1	" "
6	7·5	
7	7·0	" "
8	7·3	
9	6·8	" "
10	6·7	

The figures from the duplicate pots do not show any kind of agreement, and the deduction which can be drawn from them is that in none of the pots has a reaction been produced which would inhibit the growth of either organism. It would seem then that the incompatibility of the two organisms is due to some factor other than that of soil reaction. Since, on artificial media colonies will grow side by side on the same plate, the writers are inclined to think that the inhibitory effect of the one upon the other is not so much a question of incompatibility as of starvation. On artificial media the parasite is extremely slow in growth compared with the saprophyte, and in a soil well supplied with vegetable matter this difference in character may easily be intensified. Under such conditions it seems very possible for the parasite to be temporarily, if not permanently, starved out. Sanford (11) has shown that in artificial culture the growth of his strains of *A. scabies* was inhibited by various strains of bacteria—in some cases on account of the excessive acidity produced, and in one case to some unknown factor. It seems very feasible, indeed highly probable, that bacteria as well as saprophytic *Actinomyces* may inhibit the development of the parasitic *Actinomyces* species. The present experiments, however, do not afford any support to the theory. Thus, in the green manured pots which received inoculations of *A. scabies* alone, no diminution of scab was produced in either of the experiments. It must be remembered, however, first, that the *Actinomyces* inoculations were very heavy, and secondly, that the

bacterial flora of the soil in the pots would only slowly establish itself. Even where bacterial development was stimulated by green manure, the competition with the already-established *Actinomyces* would not be comparable to that which could occur under natural conditions. Obviously, the action of bacteria in this connection is open to the same tests as those herein described, and these would give more reliable results than can be obtained from laboratory cultures.

Although green manuring has certainly established itself in this country as a means for growing clean potatoes—in particular among gardeners and small holders—yet there appear to be a number of cases in which it has not proved effective. Doubtless some of these failures may be attributed to inefficient treatment, but others can scarcely be accounted for in this way. Sanford (12), for example, states that he failed to reduce scab with an application of green rye at the rate of $2\frac{1}{2}$ pounds per potato hill. Such a result would formerly have been very inexplicable to us, but the present work may throw some light upon it. Serious outbreaks of scab rarely occur on acid soils, and generally are most severe on neutral or alkaline soils of a light sandy nature. Where green manure is applied to the latter type of soil the conditions are those which favour a rush to maximum activity of the saprophytic microflora, and a parallel with the heavy inoculations in our own experiments is provided. Sanford gives the *p*H of the soil upon which he experimented as ranging from 4·91 to 5·4, and we suggest that his failure was due to this high initial acid reaction. He records a slight decrease in acidity as a result of green manuring, and shows that in the case of a laboratory experiment with another soil the admixture of rye caused a considerable temporary rise in *p*H value. These observations, however, do not alter the fact that vegetable matter (*e.g.* ploughed-in sod) decomposes very slowly in acid soils, and it is practically certain that under these conditions no inhibition of the scab-producing *Actinomyces* can take place. Lastly, the somewhat remote possibility should not be forgotten that in those soils where green manuring is ineffective the *Actinomyces* species or other organisms which can give a quietus to the scab-producing species may be lacking.

Summary.

1. The "preferential food theory" as an explanation of the action of green manure in the control of scab is criticised.

2. Experiments are described in which potatoes are grown in series of pots containing soil only or soil mixed with grass cuttings. In each

series the pots were inoculated with two *Actinomyces* species—the one, *A. scabies* (Thaxter) Güssow emend. M. and B., a virulent scab producer; the other, *A. praecox*, an obligate and prolific saprophyte. In Exp. 1, the inoculum of *A. scabies* was made to decrease and that of *A. praecox* to increase along the series. In Exps. 2 and 3 the inoculum of *A. scabies* was kept constant, and that of *A. praecox* was gradually increased along the series of pots.

3. In the absence of the saprophytic *Actinomyces* species, grass alone exerted no inhibitory action on scab.

4. A reduction of scab was obtained in all pots whether of soil or soil mixed with grass to which an inoculation of *A. praecox* had been given. This reduction was most marked in the case of the maximum inoculation with *A. praecox*.

5. Counts of the two *Actinomyces* species were made from soil samples taken from the pots during and after growth of the potato crops, and from these it was found that the presence of the saprophytic species had exerted a very strong depressing effect upon the parasitic species. This depressing effect was most marked after a whole season's growth as shown by Exp. 3. Here, on the millionth dilution platings from a pot inoculated with *A. scabies* alone, 225 colonies grew but no colonies of this species were found on the plates of the same dilution in the case of three pots to which inoculations of *A. praecox* had also been given.

6. The inhibitory effect of *A. praecox* on *A. scabies* is not due to the setting up of an unfavourable soil reaction but is probably caused by a starving out of the weaker organism in competition for the available food supply of the soil.

7. It is suggested that the beneficial effect of green manuring on scab under field conditions is due to similar competitive action and that in this competition the bacteria as well as the *Actinomyces* may play a part.

8. The cases in which green manuring has apparently failed to inhibit scab have been considered from this fresh point of view.

The writers wish to express their thanks to Mr H. Trefor Jones, M.Sc., Assistant Lecturer in Agricultural Chemistry in this University, for his kindness in making the estimations of the *p*H values for the soils in Exp. 3, and to Mr J. Manby for making the photographs shown in the figures.

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(7) —— —— *Ibid.* 590.
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(9) —— —— *Ibid.* 589–90.
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(11) —— *Ibid.* 541–5.
(12) —— *Ibid.* 536–41.

EXPLANATION OF PLATES XV—XVII.

Fig. 1. Exp. 1. Series *B*. Pots 1 to 4. Each horizontal row of potatoes is representative of the entire crop from one pot. Row 1 at the top is from Pot 1, and the others follow in order.

Fig. 2. Exp. 2. Series *B*. Each horizontal row of potatoes is representative of the entire crop from the duplicate pots. Row 1 at the top is from Pots 1 and 2. Row 2 from Pots 3 and 4. Row 3 from Pots 7 and 8 and row 4 from Pots 9 and 10.

Fig. 3. Exp. 3. Pots 4, 6, 8 and 10. Each group is representative of the crop from a given pot. The groups are: top, left, from Pot 4; top, right, from Pot 6; bottom, left, from Pot 8; bottom, right, from Pot 10.

(*Received December 23rd*, 1926.)

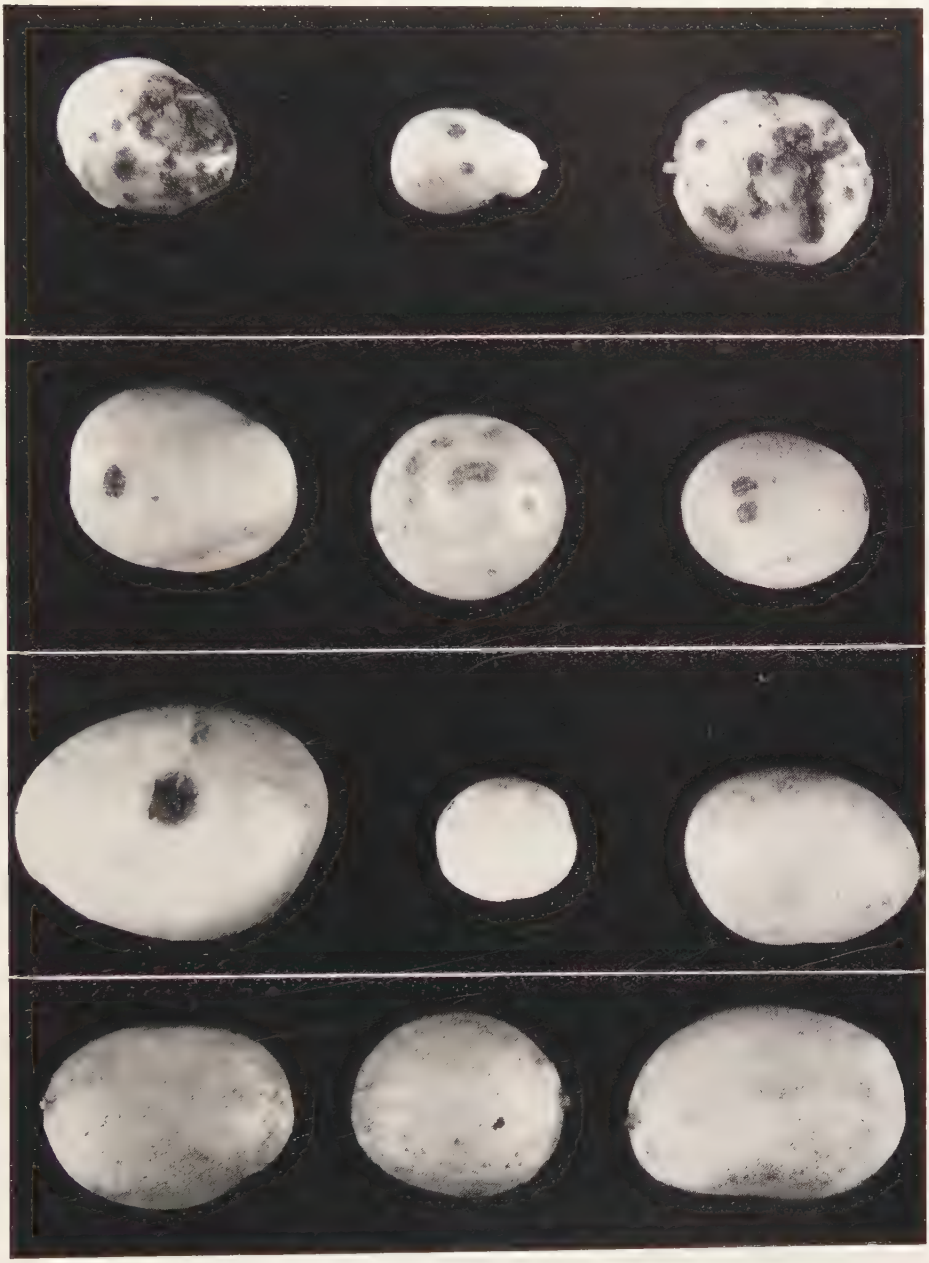

Fig. 1.

MILLARD & TAYLOR.—Inhibition of Scab by Green-manuring (pp. 202—216).

Fig. 2.

MILLARD & TAYLOR.—Inhibition of Scab by Green-manuring (pp. 202—216).

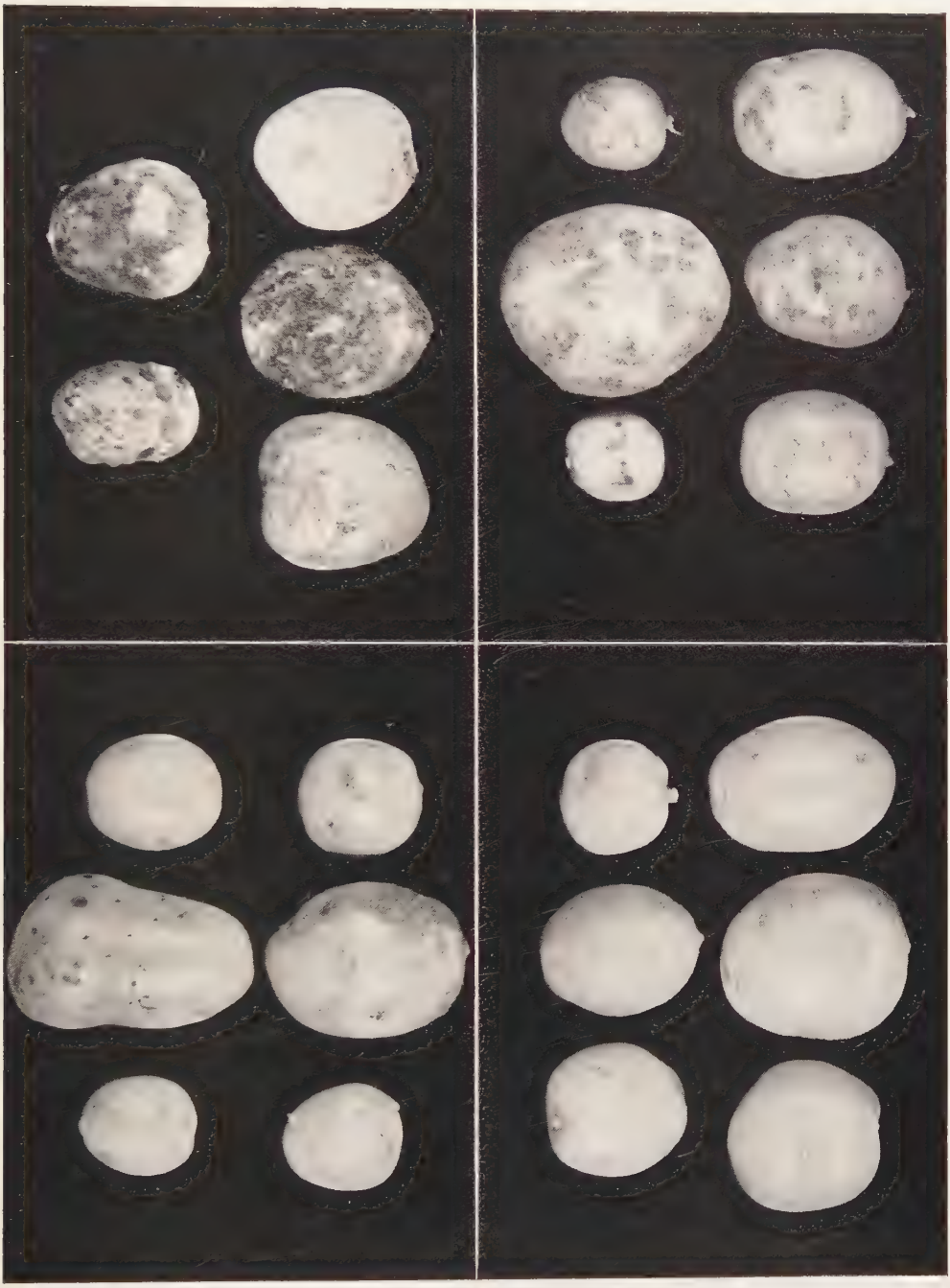

Fig. 3.

MILLARD & TAYLOR.—Inhibition of Scab by Green-manuring (pp. 202—216).

STUDIES ON CONTACT INSECTICIDES

PART V. THE TOXICITY OF THE AMINES AND *N*-HETEROCYCLIC COMPOUNDS TO *APHIS RUMICIS* L.

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(With 6 Diagrams.)

An account was given in Part III (3) of this series of papers of the insecticidal properties of the simpler chlor, nitro and hydroxy derivatives of benzene and naphthalene, compounds of a more or less acidic character. The survey has now been extended to the amines and certain of the nitrogen-heterocyclic group of organic compounds, most of which show basic properties in a greater or less degree.

In Part III definite gradations in toxicity were shown to occur amongst the nitro compounds, and some of them, such as dinitro-phenol and dinitro-*o*-cresol, proved to have marked toxic effects evidently depending upon the make-up of the molecule. We have found similar gradations amongst the compounds dealt with in this paper.

Experimental.

The method employed for the determination of insecticidal values has been dealt with in previous papers (1, 2, 3), but a brief résumé is perhaps not out of place. *Aphis rumicis* (the Black Bean Aphis) has again been used as our chief test insect, and as the result of considerable experience we are able to rear large numbers of adult wingless females of this species, during the summer months, which show little individual variation in resistance as judged by the results of many duplicate experiments. The spraying apparatus used (1) is designed to give percentage mortality figures under strictly controlled and constant conditions with regard to the amount of liquid sprayed, the pressure employed to produce the spray, and the length of time of contact. A large number of tests can be carried out rapidly and, by testing each compound at a number of concentrations, it is possible to draw diagrammatic curves from the

results, plotting percentage mortality against concentration, and thus to obtain a convenient means of comparing toxicities.

In making up the mixtures for spraying, we consistently use a 1 per cent. solution of saponin in water to ensure satisfactory wetting, and to help emulsification. When the substances to be tested are liquids, they are dissolved in or emulsified with the saponin solution. Solids insoluble in water are, whenever possible, dissolved in a small quantity of benzene and then emulsified with the saponin solution.

Control experiments, many times repeated, have shown that 1 per cent. saponin solutions, with or without the addition of benzene in amounts much greater than are used in the liquids sprayed, are without toxicity to *A. rumicis*. It has occasionally been necessary to add small amounts of other solvents; in such cases special control tests are carried out.

After spraying, the insects are placed within reach of fresh bean foliage and are examined on the two (or, if necessary, three) following days. At each examination, counts are made under four headings: "unaffected," "slightly affected," "moribund" and "dead." In the tables, except in the cases of unimportant compounds, the full data for the four categories are given, but in the diagrams, the sums of the figures in the last two categories only are plotted against the concentration. This procedure perhaps involves inadequate grading of comparative toxicities in some cases, but since "slightly affected" insects almost invariably recover and "moribund" insects almost invariably die[1], we believe that it at all events does not exaggerate, though it may tend to minimise toxicity.

Mr H. M. Morris collaborated with us in the earlier stages of this work and we wish to express our appreciation of his help and to record our thanks.

The Amines.

Quaternary compounds. Both aliphatic and aromatic amines have been tested. In the former of these groups chief attention was given to the tetramethyl and ethyl compounds, whereas amongst the aromatic compounds a general survey covering a large number of derivatives has been made.

The results obtained with the aliphatic and certain aromatic quaternary compounds are set out in Table I; the figures for tetramethyl and tetraethylammonium hydroxides and salts are plotted in Diagram 1.

[1] There is an exception in the case of compounds exhibiting anaesthetic action. These are specially noted.

Table I.

Toxicities to A. rumicis *of certain quaternary ammonium compounds and trimethylamine.*

[N = not affected; S = slightly affected; M = moribund; D = dead.]

Substance	Concentration* Gm. per 100 c.c.	Concentration* Mole per 100 c.c.	No. of tests	N %	S %	M %	D %	M and D %
Trimethylamine hydrochloride $N(CH_3)_3HCl$	5·0	·084	—	—	—	—	100	100
	2·5	·042	—	—	—	20	80	100
	1·0	·017	—	10	—	40	50	90
	0·75	·013	—	10	—	20	70	90
	0·5	·008	—	30	—	—	70	70
	0·25	·004	—	10	40	30	20	50
Tetramethylammonium hydroxide $N(CH_3)_4OH$	2·0	·022	1	—	—	—	100	100
	1·0	·011	3	3	7	3	87	90
	0·75	·0083	2	—	5	20	75	95
	0·5	·0055	3	3·5	13	23·5	60	83·5
	0·35	·0038	2	41	12	18	29	47
	0·2	·002	2	45	20	15	20	35
	0·1	·001	3	67	3	20	10	30
	0·05	·0005	3	80	6·5	3·5	10	13·5
Tetramethylammonium chloride $N(CH_3)_4Cl$	2·0	·012	1	—	—	—	100	100
	1·0	·01	3	—	—	—	100	100
	0·75	·0083	3	—	—	—	100	100
	0·5	·0055	3	7	3	10	80	90
	0·35	·0038	2	10	5	—	85	85
	0·2	·002	2	10	—	35	55	90
	0·1	·001	3	70	10	13	7	20
	0·05	·0005	3	83·5	3	10	3·5	13·5
Tetramethylammonium sulphate $(N(CH_3)_4)_2SO_4$	1·0	·011	1	—	—	10	90	100
	0·75	·0083	1	—	—	10	90	100
	0·5	·0055	1	—	20	20	60	80
	0·35	·0038	1	—	30	10	60	70
	0·2	·002	1	40	—	10	50	60
	0·1	·001	1	50	—	20	30	50
	0·05	·0005	1	90	—	—	10	10
Tetraethylammonium hydroxide $N(C_2H_5)_4OH$	2·0	·014	3	23·5	13	10	53·5	63·5
	1·5	·0102	1	40	—	10	50	60
	1·0	·007	3	30	10	13	47	60
	0·75	·0051	3	57	3	3	37	40
	0·5	·0034	3	65·5	3·5	3·5	27·5	31
	0·35	·0024	2	80	—	10	10	20
	0·25	·0017	1	80	—	10	10	20
	0·2	·0014	2	80	—	15	5	20
Tetraethylammonium chloride $N(C_2H_5)_4Cl$	2·0	·014	2	15	10	15	60	75
	1·5	·0102	1	30	—	—	70	70
	1·0	·0068	2	35	—	35	30	65
	0·75	·0051	2	25	10	15	(55)	(70)
	0·5	·0034	2	60	15	10	15	25

* The concentrations in all cases are given in terms of the respective bases.

Table I (*continued*).

[N = not affected; S = slightly affected; M = moribund; D = dead.]

Substance	Concentration: Gm. per 100 c.c.	Concentration: Mole per 100 c.c.	No. of tests	N %	S %	M %	D %	M and D %
Trimethylaniline hydroxide $C_6H_5N(CH_3)_3OH$	10·0–2·5	·065–·016	3	—	—	—	—	100
	2·0	·013	1	—	10	—	90	90
	1·0	·0066	2	16	16	5	63	68
	0·75	·0049	1	50	20	—	30	30
	0·5	·0032	2	90	—	5	5	10
Trimethyl-α-naphthylamine hydroxide $C_{10}H_7N(CH_3)_3OH$	2·0	·01	1	—	—	—	100	100
	1·0	·005	1	20	10	20	50	70
	0·75	·0037	1	20	20	—	60	60
	0·5	·0025	1	40	20	—	40	40
	0·25	·0012	1	100	—	—	—	—
Trimethyl-β-naphthylamine hydroxide $C_{10}N_7N(CH_3)_3OH$	2·76	·014	1	10	10	10	70	80
	1·38	·0078	1	10	10	10	70	80
	0·69	·0039	1	20	30	—	50	50
	0·51	·0025	1	70	—	—	30	30
	0·34	·0015	1	100	—	—	—	—
Dimethylbenzyl-phenyl-ammonium hydroxide $N(C_6H_5CH_2)(C_6H_5)(CH_3)_2OH$	2·5	·011	1	—	—	—	100	100
	1·0	·0044	1	—	—	—	100	100
	0·5	·0022	1	78	—	11	11	22

Ammonium hydroxide was not materially toxic at a concentration of 9 gm. NH_3 per 100 c.c. Ammonium chloride, ammonium sulphate, sodium chloride, sodium sulphate had little toxic effect at concentrations of 2 gm. per 100 c.c. (the highest concentration tested).

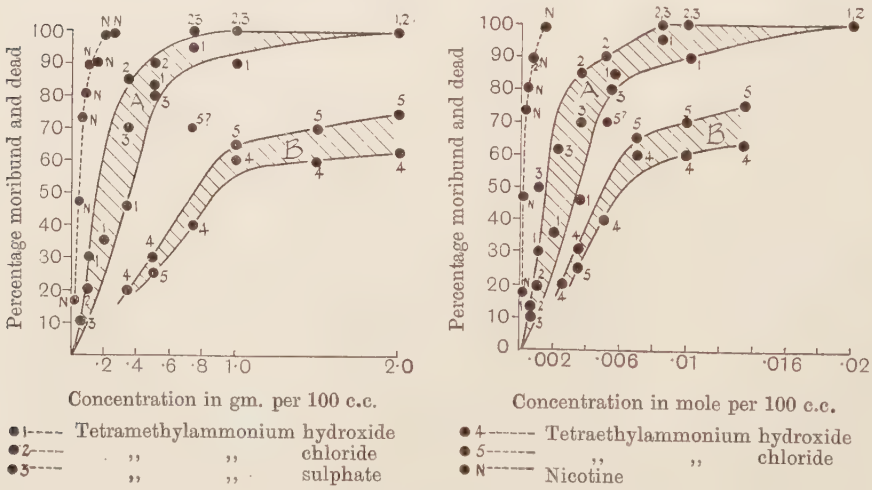


Diagram 1. Toxicities of tetramethyl- and tetraethyl-ammonium hydroxides and salts to *Aphis rumicis*.

In both table and diagram, the concentrations are expressed in grams and gram-molecules of the base per 100 c.c. of solution sprayed. The hydroxide, chloride and sulphate of tetramethylammonium and the hydroxide and chloride of tetraethylammonium were tested. Both groups of compounds proved fairly highly toxic to *A. rumicis*. In Diagram 1, the results for the tetramethyl compounds fall within the shaded area *A*, the hydroxide on the right and the chloride on the left margin, the sulphate being intermediate between the two; and those of the tetraethylammonium compounds fall in the shaded area *B*. In both cases the hydroxides appear slightly less toxic than the salts, though these differences are scarcely significant. It is noteworthy that such apparently innocuous derivatives as the chlorides of these bases should have so high a degree of toxicity. Tetraethylammonium was distinctly slower in its action than tetramethylammonium, the former showing little toxic action until after 24 hours.

It has long been known that tetramethylammonium possessed marked physiological effects upon the higher animals. The classical researches of Crum Brown and Fraser (4) in 1869 showed it to be a powerful poison with a paralysing action on the peripheral terminations of motor nerves and to have a direct action on the cerebro-spinal system. In more recent years, the researches of Barger and Dale (5) on "Chemical Structure and the Sympathomimetic Action of Amines," and of Burn and Dale (6) on the "Action of certain Quaternary Ammonium Bases," have shown that tetramethylammonium possesses certain physiological effects similar to those of nicotine, in marked contradistinction to tetraethylammonium. Burn and Dale, summarising their conclusions, say "Tetramethylammonium hydrate . . . possesses the nicotine-like action in a very intense degree, being approximately as active in its stimulation of ganglion cells as nicotine itself; it also possesses a weaker but well-marked action of the muscarine type. . . . On the ganglion cells, on the other hand, though the tetraethyl compound has no appreciable stimulating action in ordinary dosage, it exhibits the secondary paralytic phase of the nicotine-action almost as powerfully as the tetramethyl compounds."

In insecticidal tests one would expect the paralysing action to be of importance, and if this phase in the physiological action of the two compounds, tetramethyl- and tetraethylammonium, were not strongly differentiated, that there would be only insignificant differences in their respective toxicities. Our results show that, weight for weight and mole for mole, the tetramethyl compound is distinctly more toxic to

A. rumicis than the corresponding ethyl compound, indicating that in all probability the whole series of physiological effects, in which tetramethylammonium is similar to nicotine, is of importance in determining its toxicity as a contact insecticide.

Richardson and Smith (7) also tested the chlorides of these bases upon *A. rumicis*, using a different technique, and determined the minimum concentration killing 95 per cent. They found the tetramethylammonium compound to be almost seven times as toxic as the tetraethyl derivative mole for mole.

The relatively high toxicity of the tetramethyl compounds as compared with the corresponding ethyl derivatives is not readily explained, and there is little in the literature which seems helpful. In the theory of Overton (8) and Meyer (9), an attempt is made to correlate narcotic action with the partition coefficients of compounds (*i.e.* the ratio of solubility in lipoid or oil to solubility in water). Some work on the possibility of a relationship between partition coefficients and insecticidal action is discussed in a later paper in connection with another group of compounds—the fatty acids. In the absence of direct experimental work on these lines on the amines, it is impossible to say whether Overton and Meyer's theory would offer any help in accounting for the difference in toxic action between tetramethyl- and tetraethylammonium. The theory has been subjected to much criticism, and, expressed in its simplest form, it seems unlikely to be helpful, for it is difficult to believe that any close correlation exists between the partition coefficients and the insecticidal action of these substances. Harvey (10) has shown that weak bases penetrate tissues of marine organisms more rapidly than strong bases, and that tetraethylammonium hydroxide, a highly dissociated base, cannot penetrate sea-urchin's eggs and is less toxic than mono-ethylamine hydroxide, a slightly dissociated base. The effect of tetramethylammonium hydroxide is not discussed in his paper. According to Harvey, the degree of dissociation can act in two countervailing ways: the higher it is the lower the penetration power, and in consequence the toxic action; but provided penetration does take place, a more highly dissociated base shows a greater toxicity than one of a lower degree of dissociation. In the case of tetramethyl- and tetraethylammonium, however, both bases are highly dissociated.

It is a matter of common knowledge that methyl and ethyl groups, especially in the alcohols, differ in their physiological effects, and the investigations of Flusin (11) on the penetration of the pig's bladder by

certain alcohols showed that, whereas methyl alcohol penetrates readily, this membrane is almost impermeable to ethyl alcohol. Thus, despite the close similarities between the methyl and ethyl groups in both chemical and physical properties, we are forced to the conclusion that both biochemically and biophysically they must react with some element of the living organism in different ways. The difficulty of accounting for the differences in the physiological action of tetramethyl- and tetraethylammonium is summed up by Dale(12) in the words "we are driven back on a conception of some peculiar conformation of the reactive surface to which the methyl groups are adjusted which the ethyl groups will not fit," and they apply equally well to the differences shown by the compounds in their insecticidal action.

In Table I the results obtained with trimethylamine hydrochloride are also given; it proved only slightly less toxic than tetramethylammonium chloride, a result which agrees with that of Richardson and Smith (*loc. cit.*). In addition, certain other quaternary compounds, such as phenyl-trimethylammonium hydroxide, and α-naphthyl-trimethylammonium hydroxide are placed here for purposes of comparison. Although the experiments with these compounds were only of a preliminary nature, they showed that methyl cannot be replaced by the aromatic radical phenyl, or α-naphthyl, with any advantage, the resulting compounds being on the whole less toxic both weight for weight and mole for mole than tetramethylammonium hydroxide. The compound dimethylbenzylphenylammonium hydroxide is of the same order of toxicity as tetramethylammonium hydroxide when molecular comparisons are made, but it is much less toxic when compared on a gram for gram basis.

Thus, so far as we have been able to test this series, the tetramethylammonium radical is unique in its toxic action.

The aromatic amines (*aniline derivatives*). Table II and Diagram 2 show the results obtained with some aromatic amines.

A large number of the more readily available amines were tested. It would be impossible to graph in any clear way the whole of the results obtained, and the tables have been shortened in many cases, by giving only the outstanding data. In Diagram 2 a shaded area is given within which practically all the aliphatic aniline derivatives fall. The isobutyl and iso-amyl anilines appear on the margin to the left, methyl aniline and toluidine fall to the right of this area, aniline itself being well outside to the right; dimethylaniline practically bisects the area. The labour required to differentiate further between these compounds did not seem

Table II.

Toxicities to A. rumicis *of the aromatic amines.*

[N = not affected; S = slightly affected; M = moribund; D = dead.]

Substance	Concentration: Gm. per 100 c.c.	Concentration: Mole per 100 c.c.	No. of tests	N %	S %	M %	D %	M and D %
Aniline	10·0	·11	1	—	—	—	100	100
$C_6H_5NH_2$	5·0	·053	1	—	10	20	70	90
	4·0	·042	1	30	20	10	40	50
	3·0	·032	1	70	10	10	10	20
	2·0	·021	1	80	10	—	10	10
	1·0	·011	1	90	10	—	—	—
Methylaniline	5·0–2·5	·047–·023	5	—	—	—	—	100
$C_6H_5NH(CH_3)$	2·0	·019	1	—	—	70	30	100
	1·0	·0093	2	50	10	15	25	40
	0·75	·007	1	80	—	20	—	20
	0·5	·0047	2	95	—	5	—	5
	0·25	·0023	2	90	10	—	—	—
Dimethylaniline	4·0	·032	2	—	—	—	100	100
$C_6H_5N(CH_3)_2$	3·0	·024	2	—	—	15	85	100
	2·5	·02	1	—	—	—	100	100
	2·0	·016	2	5	—	32	63	95
	1·0	·008	3	30	—	46·7	23·3	70
	0·5	·004	3	80	—	6·7	13·3	20
	0·25	·002	3	90	—	3·3	6·7	10
Ethylaniline	5·0–2·0	·041–·016	4	—	—	—	—	100
$C_6H_5NH(C_2H_5)$	1·0	·0082	1	30	—	50	20	70
	0·5	·0041	1	67	—	11	22	33
	0·25	·002	1	70	10	20	—	20
Diethylaniline	5·0–2·0	·034–·013	4	—	—	—	—	100
$C_6H_5N(C_2H_5)_2$	1·0	·0067	1	50	—	10	40	50
	0·5	·0033	1	80	—	—	20	20
	0·25	·0016	1	89	—	—	11	11
o-Nitraniline	1·0	·0072	1	—	—	—	100	100
$C_6N_4NH_2NO_2$	0·75	·0054	2	—	—	—	100	100
	0·5	·0036	2	—	—	10	90	100
	0·25	·0018	2	10	—	30	60	90
	0·125–0·1	·009–·0072	2	65	—	10	25	35
	0·5	·0036	1	70	20	10	—	10
Diphenylamine	1·0	·0059	3	—	—	20	80	100
$(C_6H_5)_2NH$	0·75	·0045	2	5	—	35	60	95
	0·5	·003	2	—	5	35	60	95
	0·35	·0021	1	10	10	10	70	80
	0·25–0·2	·0015–·0012	2	45	5	35	15	50
	0·1	·00059	2	48	—	38	14	52
Triphenylamine $(C_6H_5)_3N$	1·0	·0041	—	Not appreciably toxic				
Methyldiphenylamine	1·0	·0054	1	—	—	—	100	100
$(C_6H_5)_2NCH_3$	0·75	·004	1	—	—	—	100	100
	0·5	·0027	1	—	—	20	80	100
	0·35	·0019	1	50	—	20	80	100
	0·2	·0011	1	60	—	—	40	40
	0·1	·00054	1	70	—	20	10	30

Table II (*continued*)

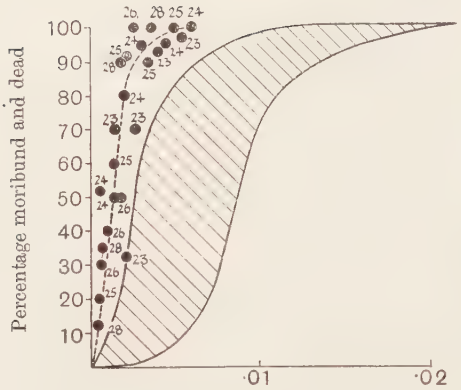
[N = not affected; S = slightly affected; M = moribund; D = dead.]

Substance	Concentration: Gm. per 100 c.c.	Concentration: Mole per 100 c.c.	No. of tests	N %	S %	M %	D %	M and D %
Benzylamine $(C_6H_5CH_2)NH_2$	2·0	·019	—	Not appreciably toxic				
Dibenzylamine $(C_6H_5CH_2)_2NH$	5·0–1·0	—	6	—	—	—	100	100
	0·5	·0025	1	—	—	—	100	100
	0·25	·00125	1	—	30	40	30	70
Tribenzylamine $(C_6H_5CH_2)_3N$	2·0	·007	—	Not appreciably toxic				
Benzylaniline C_6H_5 \ NH / $C_6H_5CH_2$	1·0	·0054	4	2·5	—	30	67·5	97·5
	0·75	·004	3	7	—	50	43	93
	0·5	·0027	3	13	17	37	33	70
	0·35	·0019	1	30	40	30	—	30
	0·2	·0011	1	50	30	10	10	20
	0·1	·0054	3	57	27	6	10	16
Dibenzylaniline $(C_6H_5CH_2)_2$—N / C_6H_5	1·0	·004	—	Not appreciably toxic				
Methylbenzylaniline C_6H_5 \ $C_6H_5CH_2$-NCH_3	1·0	·005	1	—	—	10	90	100
	0·75	·0037	1	—	10	10	80	90
	0·5	·0025	1	10	—	20	70	90
	0·25	·0012	1	10	30	10	50	60
	0·1	·0005	1	30	50	10	10	20

The following were tested at a number of concentrations and were found to secure 90–100 per cent. of deaths at strengths ranging from 1–2·5 gm. per 100 c.c.; the points representing the toxicities at different concentrations all fall within or very near the shaded area in Diagram 2: *o*- and *p*-toluidine, dimethyl-*o*- and *p*-toluidine, *o*-, *m*- and *p*-xylidine, di-*n*-propylaniline, *iso*- and di-*iso*propylaniline, *iso*butylaniline, *iso*- and di-*iso*amylaniline, dibenzylaniline, *o*- and *p*-anisidine, *o*- and *p*-phenetidine, monomethylaniline nitrosamine, nitrosomonomethylaniline nitrosamine, *p*-nitrosodimethylaniline.

The phenylenediamines (*o*, *m* and *p*) were not toxic at a concentration of 1 per cent. Aminodiphenylamine was not appreciably toxic at 1 per cent. Triphenylamine was much less toxic than diphenylamine, having little effect at or below a concentration of 1 per cent.

The *m*- and *p*-nitranilines, owing to their relatively low solubility in benzene, were difficult to work up into a form suitable for spraying, but were not appreciably toxic at the strengths tested. The following compounds were also tested: β-phenylhydroxylamine, hydrazobenzene, azobenzene, azotoluene, azoxybenzene, azoxytoluene. β-phenylhydroxylamine and azoxybenzene secured 90–100 per cent. of deaths at concentrations ranging from 0·75 to 1·0 gm. per 100 c.c.; this was also the case with diazoaminobenzene which was more toxic than aminoazobenzene.

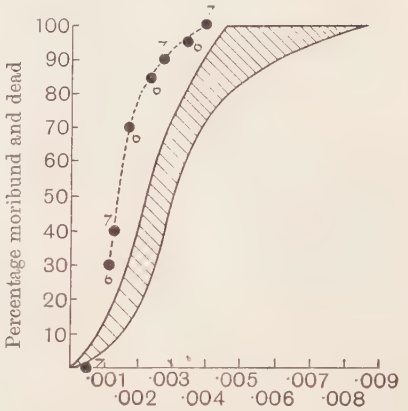


Concentration in mole per 100 c.c.

● 23 --- Benzylamine
● 24 --- Diphenylamine
● 25 --- Methylbenzylaniline
● 26 --- Methyldiphenylamine
● 27 --- Dibenzylamine
● 28 --- *o*-nitraniline

The other aromatic aniline derivatives fall within the shaded area, with the exception of aniline, which falls outside to the right

Diagram 2. Toxicities of the aromatic amines to *Aphis rumicis*.



Concentration in mole per 100 c.c.

● 6 --- Phenyl-α-naphthylamine
● 7 --- Nitro-α-naphthylamine (crude)

α-naphthylamine, methyl-, dimethyl-, and ethyl-α-naphthylamines fall within the shaded area

Diagram 3. Toxicities of α-napthylamine derivatives to *Aphis rumicis*

worth while. The main deduction to be drawn is that in the case of aniline the substitution of methyl, methoxyl and ethoxyl groups in the benzene ring or of aliphatic groups on the nitrogen do not give rise to any great increases in toxicity although all are more toxic than the original compound. On the other hand, aromatic nuclei, substituted on the nitrogen, give rise to marked increases in toxicity over the parent substance; thus diphenylamine, methyldiphenylamine, benzyl-aniline, methylbenzylaniline are more toxic than aniline or any of the aliphatic anilines, both weight for weight and mole for mole. The phenyl group appears to be of greater significance than the benzyl group in this respect, but the points on the diagram come so near together that the toxicities of all four of these substances can be fairly well represented by one curve. Triphenylamine proved less toxic than diphenylamine and dibenzyl- and benzalaniline less toxic than benzylaniline.

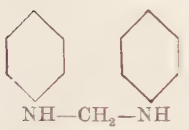
The results obtained with the benzylamine derivatives are particularly interesting, for they show toxic relationships *inter se* of the same kind as those shown by the phenylamines. Dibenzylamine is considerably more toxic than either benzylamine or tribenzylamine.

This is analogous to the effects obtained with the phenylamines and benzylanilines; *i.e.* if in each of these series, ammonia (NH_3) is taken as the basic radical, the compounds in which two hydrogen atoms are replaced by aromatic nuclei are more toxic than those in which one or three are substituted. We have therefore the following orders[1] of toxicity:

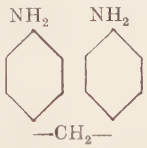
phenylamine(aniline) < diphenylamine > triphenylamine.
phenylamine(aniline) < benzylaniline > dibenzylaniline.
benzylamine < dibenzylamine > tribenzylamine.

These relationships raise the question whether the last hydrogen attached to the nitrogen which remains unsubstituted in these compounds has not a definite significance in determining the toxicity. This hydrogen, however, can be replaced by methyl without loss of toxic properties, methyl-diphenylamine and methyl-dibenzylaniline have insecticidal properties of the same order as diphenylamine.

The substitution of a second amino group in the *o*, *m* or *p* position, or of chlorine or hydroxyl groups, in the aniline ring produces no marked increase in toxicity, and bases of the type methylene-diphenyl-di-imide



and diamino-diphenylmethane



show no appreciable toxicity at a concentration of 1·0 per cent. This is also true of benzylethylaniline, formyl-diphenylamine, the benzidine and tolidine bases, anhydro-formaldehyde-aniline and anhydro-formaldehyde-*o*-toluidine.

The introduction of a nitro group in the *o*-position to the amino group in aniline has a pronounced effect on toxicity. *o*-Nitraniline is one of the most toxic derivatives of aniline tested. On a gram-molecular scale the points happen to fall close to the curve showing the toxicity of diphenylamine (see Diagram 4). The *m*- and *p*-nitro derivatives were not sufficiently soluble to permit of testing at any concentration at which they showed toxicity.

The nitroso compounds present few points of interest; the nitrosamine of methylaniline, the nitrosamine of nitroso-methylaniline and *p*-nitroso-dimethylaniline were toxic at concentrations of about 0·75–1·0 per cent.

[1] The nitrophenols and nitrocresols show effects of a similar type: mono-nitrophenols < 2 : 4-dinitrophenol > 2 : 4 : 6-trinitrophenol (*Ann. App. Biology*, 1925, xii, 218).

Naphthylamine derivatives. In Table III and Diagram 3 are set out the results obtained with α- and β-naphthylamine and a few of their simple derivatives.

Table III.

Toxicities to A. rumicis *of some naphthylamine derivatives.*

[N = not affected; S = slightly affected; M = moribund; D = dead.]

Substance	Concentration: Gm. per 100 c.c.	Concentration: Mole per 100 c.c.	No. of tests	N %	S %	M %	D %	M and D %
α-Naphthylamine	1·0	·007	1	—	—	—	100	100
$C_{10}H_7NH_2$	0·75	·005	1	10	—	—	90	90
No solvent: Fine suspensoid	0·5	·0035	1	10	—	—	90	90
	0·375	·0026	1	30	—	—	70	70
Ditto coarse suspensoid				Not materially toxic at 1 %				
Ditto benzene used as solvent*	1·0	·007	2	10	—	—	90	90
	0·75	·005	2	15	—	5	80	85
	0·5	·0035	3	26	10	6	58	64
	0·35	·0025	1	50	—	20	30	50
	0·25	·0018	2	90	—	5	5	10
	0·2	·0014	1	80	10	—	10	10
	0·1	·0007	2	75	15	5	5	10
Methyl-α-naphthyl-amine	1·0	·0064	2	—	—	10	90	100
	0·75	·0048	2	—	—	11	89	100
$C_{10}H_7NH(CH_3)$	0·5	·0032	2	20	5	25	50	75
	0·35	·0021	1	60	—	—	40	40
Dimethyl-α-naphthylamine	1·0	·0058	1	—	—	—	100	100
	0·75	·0044	1	—	—	—	100	100
$C_{10}H_7N(CH_3)_2$	0·5	·0029	2	25	10	10	55	65
	0·35	·002	1	60	20	—	20	20
Diethyl-α-naphthyl-amine	1·0	·005	1	—	—	—	100	100
	0·75	·0037	2	—	5	—	95	95
$C_{10}H_7N(C_2H_5)_2$	0·5	·0025	3	40·5	4	7·5	48	55·5
	0·25	·0013	2	70	—	—	30	30
Phenyl-α-naphthyl-amine	1·0	·0046	2	—	—	40	60	100
	0·75	·0034	2	5	—	40	55	95
$C_{10}H_7NH\text{-}C_6H_5$	0·5	·0023	2	5	10	40	45	85
	0·35	·0017	1	30	—	60	10	70
	0·25	·0011	1	50	20	—	30	30

Ethyl-α-naphthylamine gave irregular results but a mortality of 90–100 per cent. was shown at concentrations between 1 and 2 per cent. Amyl-α-naphthylamine (containing some di-derivative) and nitroso-α-naphthylamine (commercial) secured a mortality of 80–100 per cent. at concentrations of 0·5–0·75 per cent. Di- and tetrahydro-α-naphthylamine (*ar*), dihydro-dimethyl-α-naphthylamine gave mortalities between 80 and 100 per cent. at concentrations between 0·75 and 1·0 per cent.

β-Naphthylamine and its derivatives were less toxic than α-naphthylamine and its derivatives. β-Naphthylamine, dimethyl-β-naphthylamine, phenyl-β-naphthylamine, tetrahydro-β-naphthylamine (*ac*) were not completely toxic at concentrations of 1·0 per cent.

1 : 2-naphthalene-diamine was not toxic at a concentration of 1·0 per cent.

* Benzene used as a solvent at the rate of 2 c.c. per cent. of the spray fluid for the highest concentration (1 per cent. α-naphthylamine), and in proportion at the lower concentrations.

Figures in Table III show that α-naphthylamine is rather more toxic than β-naphthylamine.

The points expressing the toxicities of a number of derivatives such as methyl-, dimethyl-, ethyl- and diethyl-α-naphthylamines fall within the shaded area of Diagram 3. All the β-naphthylamine derivatives tested were less toxic than the corresponding α-compounds.

α-Naphthylamine is also more toxic, weight for weight and mole for mole, than aniline; in general, however, radicals which when substituted in the aniline molecule produce distinct increases in toxic action, have a less marked effect when substituted in α-naphthylamine. α-Naphthylamine was tested both as a fine suspension and as an emulsion of its benzene solution in saponin and water. The preparation of a fine suspension of α-naphthylamine in 1 per cent. saponin solution is a matter of some difficulty, and it is usually unstable owing to aggregation of the particles and the growth of crystals. The finely suspended solid was more toxic than the emulsion of the benzene solution in saponin solution, the figures for the former falling outside the left margin of the shaded area in Diagram 3 and those for the latter lying on the right. The results for the fine suspension are based, however, on one experiment only, as, except on one occasion, we were unable to maintain the suspension for a sufficient length of time to carry out the trials.

As in the case of the corresponding aniline derivatives, phenyl-α-naphthylamine and nitro-α-naphthylamine[1] were more toxic than the other α-naphthylamine derivatives tested. The results are expressed on Diagram 3 by the dotted curve.

For purposes of comparison the results obtained with a few of the more toxic compounds of aniline and α-naphthylamine are plotted together in Diagram 4.

Diphenylamine is appreciably more toxic than phenyl-α-naphthylamine, but nitraniline is rather more toxic than the crude sample of nitro-α-naphthylamine, when comparisons are made between concentrations on a gram percentage basis. Diagram 4 shows that, mole for mole, however, one curve represents with a fair degree of accuracy the toxicities of all these derivatives. α-Naphthylamine itself is more toxic than aniline, both weight for weight and mole for mole; the introduction of the naphthyl group into the ammonia radical (NH_3)[2] affects toxicity more than the introduction of a phenyl group. The substitution of a

[1] The nitro-naphthylamine was a technical product, which could not be purified readily by recrystallisation.

[2] Ammonia itself in solution is only very slightly toxic.

phenyl nucleus, however, in the amino group of aniline increases toxicity proportionally to a greater extent than its substitution in the amino group of α-naphthylamine.

Looking at these relationships between toxicity and structure from the standpoint of substitution in the respective hydrocarbon nuclei, we find that the introduction of the amine group into benzene or into naphthalene materially increases toxicity; but the influence of the amino group does not seem to be additive, since the introduction of a second NH_2 does not give rise to any great increase in toxicity; indeed,

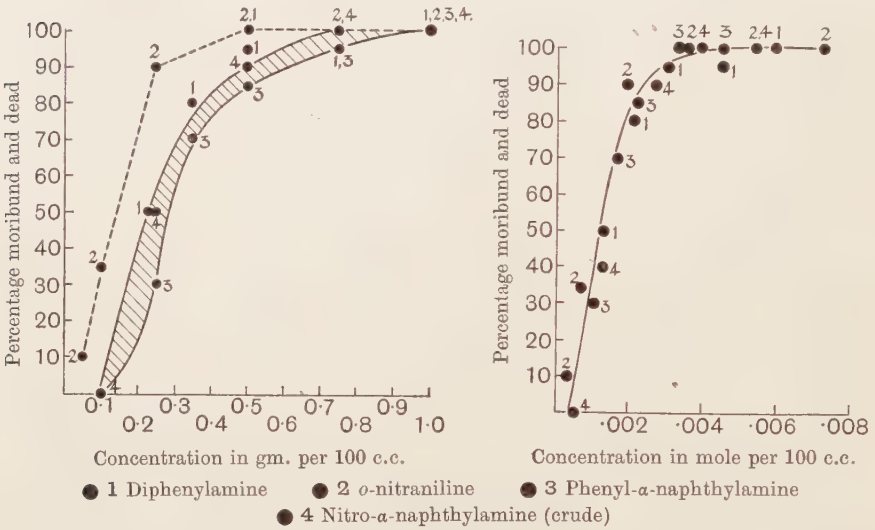


Diagram 4. Toxicities of diphenylamine, *o*-nitraniline, phenyl-α-naphthylamine and nitro-α-naphthylamine (crude) to *Aphis rumicis*

the α-β-diamine of naphthalene is less toxic than α-naphthylamine. The NH_2 group in the aromatic amines may act by increasing the affinity for oxygen and there may be an optimum degree of reactivity and, if a compound absorbs oxygen too rapidly, its toxic action may be destroyed before reaching a vital point of the insect. Thus the diamines which are less stable than the monamines may well show a less toxic action because they are oxidised to innocuous derivatives shortly after spraying.

Hydrogenation of the naphthylamine molecule does not produce any marked increase in toxicity; *ar*-dihydro and *ar*-tetrahydro-α-naphthylamine and the *ac*-tetrahydro-β-naphthylamine are not more toxic than α-naphthylamine.

Nitrogen-heterocyclic Compounds.

This group of compounds is of considerable importance from an insecticidal point of view. It includes nicotine; and in recent years the dipyridyls prepared by C. R. Smith (13) have been shown by Richardson (14) to have a high potency as contact insecticides; pyridine and its homologues have also occasionally been employed for this purpose. An examination was therefore undertaken of the toxic properties of the more commonly occurring members of this series.

Nicotine (β-pyridyl-α-N-methylpyrrolidine). As a contact insecticide, nicotine has been in common use for many years and is now regarded as the standard material for this type of work; for that reason we refer to it first among the heterocyclic compounds.

Much work has been done by various investigators on the physiological action of nicotine upon the higher animals, and it has been shown to have certain characteristic effects on the nervous system. McIndoo (15) showed that spray solutions of nicotine neither pass into the tracheae nor penetrate the integuments of insects, whereas the vapour does so and is there condensed; it is however found distributed to all the tissues. In insects, as well as in the higher animals, nicotine kills by paralysis of the nervous system, travelling along the central cord from the abdomen to the brain. McIndoo states that it gives rise to the same physiological changes as those caused by deprivation of oxygen.

It was convenient in the earlier part of this work to use pure nicotine as a standard of comparison and a large number of tests have been

Table IV.

Toxicity of nicotine to A. rumicis.

[N = not affected; S = slightly affected; M = moribund; D = dead.]

Substance	Concentration: Gm. per 100 c.c.	Concentration: Mole per 100 c.c.	No. of insects	N %	S %	M %	D %	M and D %	M and D % allowing for control
Nicotine	·25	·0015	108	0·9	—	1·8	97·2	99·0	99·0
	·2	·0012	210	1·0	0·5	5·7	92·9	98·6	98·5
	·15	·0009	143	7·7	2·1	9·1	81·1	90·2	89·8
	·1	·0006	170	7·1	2·9	12·9	77·1	90·0	89·6
	·08	·0005	149	6·0	12·1	22·8	59·1	81·9	81·1
	·06	·00035	145	19·8	4·7	17·1	57·4	74·5	73·4
	·04	·00025	148	38·5	11·5	11·5	38·5	50·0	47·8
	·02	·0001	143	76·9	2·8	9·1	11·2	20·3	16·8
	·01	·00006	210	79·5	3·3	4·3	12·9	17·3	13·7
	·005	·00003	145	91·7	0·7	1·4	6·2	7·6	3·6
	·0025	·00001	148	93·9	—	1·4	4·7	6·1	2·0
Control	—	—	142	95·1	0·7	0·7	3·5	4·2	—

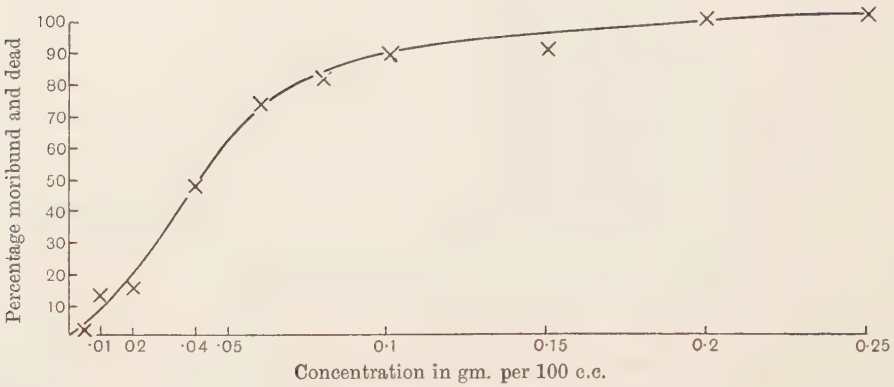


Diagram 5. Toxicity of nicotine to *Aphis rumicis* (allowing for controls). Average for three years' results.

carried out in the course of our experiments, the results being set out in Table IV and Diagram 5.

These figures are the average of the results obtained during three years, the total number of insects used at each concentration being over 100. Under the conditions of the experiments, in which the insects used were all at the same stage (adult apterous agamic females), nicotine is probably less effective than if aphides, including all stages of development, were used. There was some evidence, during the course of the experiments, that external meteorological conditions had an effect upon toxicity, but the data on this point are not sufficiently full to warrant discussion at this stage.

Our results indicated that, on the average, a concentration of about 0·1 per cent. of this alkaloid killed 90–100 per cent. of the adult insects which is in fairly close agreement with the opinion of Pickering (16) that a concentration of 0·075 per cent. was effective against aphis, apple psylla, etc. In small scale field-trials on beans heavily infested with all stages of *A. rumicis*, we have found that, in general, lower concentrations of nicotine than the above established a good control. Thus, with soft soap as a spreader, a concentration of 0·025 per cent. gave good control, 0·05 per cent. good to complete control, and 0·1 per cent. complete control.

Pyrrole, pyrrolidine, pyridine and piperidine. The results obtained for these compounds are given along with the other members of this series tested, in Table V.

They are of special interest owing to their close relationship to nicotine.

Table V.

Toxicities to A. rumicis *of some N-heterocyclic compounds.*

[N = not affected; S = slightly affected; M = moribund; D = dead.]

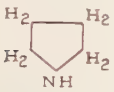
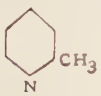
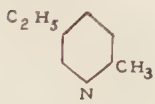
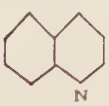
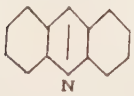
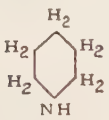
Substance	Concentration: Gm. per 100 c.c.	Concentration: Mole per 100 c.c.	No. of tests	N %	S %	M %	D %	M and D %
Pyrrole	Shows an anaesthetic action. Not materially toxic at a concentration of 10·0 %							
Pyrrolidine 	3·5–2·5	·049–·035	2	—	—	5	95	100
	1·0	·014	1	—	—	—	100	100
	0·75	·011	1	60	10	10	20	30
	0·5	·007	1	100	—	—	—	—
Pyridine	10·0	·13	2	—	—	50	50	100
	7·5	·095	1	—	—	100	—	100
	5·0	·063	2	50	—	35	15	50
	2·5	·031	2	58	10·5	21	10·5	31·5
	1·0	·013	2	65	10	20	5	25
	0·75	·0095	1	90	—	10	—	10
	Has an anaesthetic effect							
α-Picoline 	10·0	·11	1	—	—	40	60	100
	7·5	·081	1	—	—	20	80	100
	5·0	·054	1	—	20	50	30	80
	2·5	·027	1	60	20	—	20	20
Lutidine $(CH_3)_2C_5H_3N$	10·0–5·0	·083–·047	3	—	—	—	—	100
	2·5	·023	2	15	—	10	75	85
	1·0	·0093	2	30	—	10	60	70
	0·75	·007	1	90	—	—	10	10
	0·5	·0047	2	95	—	5	—	5
Collidine (2-methyl-5-ethyl-pyridine) 	5·0	·041	1	—	—	—	100	100
	3·5	·031	1	—	40	40	20	60
	2·5	·021	1	20	60	10	10	20
	1·0	·0083	1	70	10	—	20	20
Quinoline 	5·0–2·5	·039–·019	3	—	—	—	—	100
	1·5	·011	1	—	—	40	60	100
	1·0	·0077	3	6·5	6·5	30	57	87
	0·75	·0058	2	25	5	45	25	70
	0·5	·0039	3	14	18	3·5	64·5	68
	0·25	·0019	3	57	10	7	26	33
	0·1	·00077	2	95	—	—	5	5

Table V (*continued*)

[N = not affected; S = slightly affected; M = moribund; D = dead.]

Substance	Concentration: Gm. per 100 c.c.	Concentration: Mole per 100 c.c.	No. of tests	N %	S %	M %	D %	M and D %
Isoquinoline	2·5	·019	1	—	—	10	90	100
	1·5	·011	1	—	—	—	100	100
	1·0	·0077	2	5	—	20	75	95
	0·75	·0058	2	15	5	30	50	80
	0·5	·0039	2	60	10	10	20	30
	0·25	·0019	2	90	10	—	—	—
Acridine 	1·0	·0056	1	—	10	50	40	90
	0·75	·0042	1	—	—	100	—	100
	0·5	·0028	1	—	10	70	20	90
	0·25	·0014	1	10	10	80	—	80
	0·1	·00056	1	80	—	20	—	20
	0·05	·00028	1	90	10	—	—	—
Piperidine 	5·0–3·5	·059–·041	2	—	—	25	75	100
	2·5	·029	2	—	—	45	55	100
	1·0	·012	2	40	25	5	30	35
	0·75	·0088	1	90	—	—	10	10
	0·5	·0059	2	70	15	10	5	15
	0·25	·0029	2	90	10	—	—	—
Benzylpyridine* distilling 276°–278° C.	2·5–1·0	·015–·0059	2	—	—	—	—	100
	0·5	·003	3	—	—	13	87	100
	0·35	·002	2	5	5	10	80	90
	0·25	·0015	1	—	—	70	30	100
	0·2	·0012	2	35	—	10	55	65
	0·1	·00059	2	45	—	20	35	55
	0·075	·00044	1	60	—	—	40	40
	0·05	·0003	1	80	—	—	20	20
Crude benzylpyridine distilling in steam	0·5	—	2	—	—	5	95	100
	0·35	—	2	—	—	15	85	100
	0·2	—	2	—	—	25	75	100
	0·1	—	2	15	25	20	40	60
	0·075	—	2	20	25	10	45	55
	0·05	—	2	5	35	25	35	60
	0·025	—	1	20	40	20	20	40
Pyridine-N-benzyl chloride $C_5H_5NC_7H_7Cl$	5·0–2·5	—	2	—	—	—	100	100
	1·0	—	3	—	—	42	58	100
	0·75	—	2	30	—	35	35	70
	0·5	—	2	30	5	20	45	65
	0·35	—	1	40	40	20	—	20

N-methyl- and *iso*propyl-piperidines were of the same order of toxicity as piperidine. *Iso*amyl- and benzyl-piperidine were slightly more toxic than piperidine. Quinaldine and tetrahydroquinaldine secured a mortality between 80 and 100 per cent. at concentrations of 0·5–1·0 per cent. 2 : 4-Dimethylquinoline, chinosol sulphate and base, and acriflavine were not completely toxic at 1·0 per cent. The choline bases of pyridine and their hydrochlorides secured a mortality of 80–100 per cent. at concentrations of 2·5 per cent. Triacetonamine was not completely toxic at a concentration of 2·5 per cent.

α-amino-pyridine and 2 : 6-diamino-pyridine were not toxic at concentrations of 1 gm. per 100 c.c.

* The fractions of benzyl pyridine distilling from 278° to 304° C. differed little in toxicity from the fraction distilling 276° to 278° C.

Neither pyrrole not pyridine have any marked toxic action. Pyrrole shows an anaesthetic effect on *A. rumicis* and at such considerable concentrations as 10·0 per cent. the majority of insects sprayed recovered. We found pyridine to be slightly more poisonous than pyrrole, nevertheless its toxicity is so slight as to render it almost worthless as an insecticide. Brunton and Tunnicliffe (18) have shown that although pyridine has some slight effect upon the sensory apparatus, respiration and heart action of frogs, it can be regarded as comparatively non-poisonous. It is curious that it should have been found (17) to be highly toxic in the vapour phase to certain mites and their eggs, to which it seems a highly specific poison.

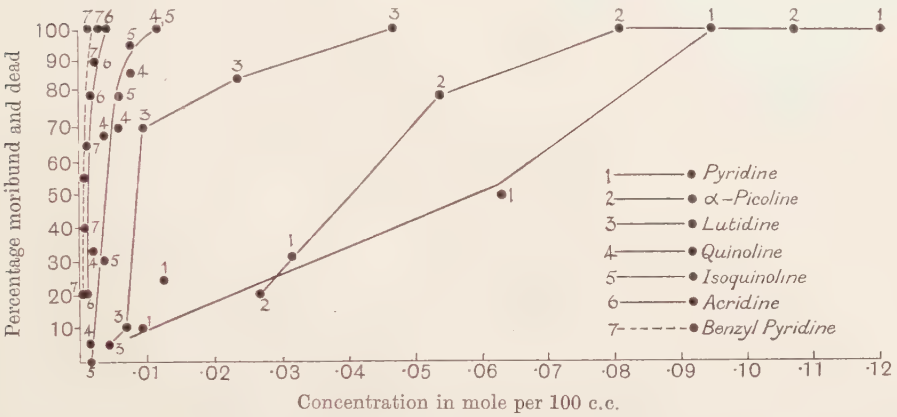


Diagram 6. Toxicity of certain heterocyclic derivatives to *Aphis rumicis*.

The effect of hydrogenation on the toxicity of both pyrrole and pyridine is considerable, pyrrolidine and piperidine being found to be relatively highly toxic when compared with their parent substances. This may be due to increased reactivity and a consequent increased effect upon the nervous system. It has been shown (cf. Spiegel (19)) that pyrrolidine and piperidine have a paralytic action on both central and peripheral nerves. Both are however much less toxic to insects than nicotine, which undoubtedly owes its high potency to its molecular make-up taken as a whole; the attachment of the pyrrolidine nucleus in the β-position of the pyridine ring, and the presence of an asymmetric carbon atom may be significant.

The results obtained with pyridine and various other members of this group, ranging from pyridine to acridine, are expressed in Table V and Diagram 6. Certain other compounds such as the naphthacridines

were prepared, but were too slightly soluble to be tested in any thorough way. They did not appear to possess any marked toxic properties.

In Diagram 6, the concentrations are plotted as fractions of a gram-molecule per 100 c.c. against the percentage of moribund and dead. There is a steady increase in toxicity as the molecular weight increases: pyridine < picoline < lutidine < quinoline and isoquinoline < acridine. We were unable to secure a sample of trimethylpyridine (collidine); its isomeride ethylmethylpyridine was slightly less toxic than lutidine.

M'Kendrick and Dewar (20) have shown that with various types of animals, the physiological action on the sensory nerves is augmented by the introduction of aliphatic side chains into compounds of this group, and that in the series pyridine, picoline, lutidine, etc., there is an increase in the effect in passing up the series. In their action upon insects the same order is observed. Whether this is due to an increase in molecular weight, or to change in some physical property in correlation with the increased bulk of the molecule, has not up to the present been ascertained.

The toxicities of quinoline and isoquinoline are identical and apparently the points at which the pyridine and benzene nuclei are fused in these compounds are unimportant in determining toxicity to insects.

The substitution of a benzyl group has a pronounced effect on the toxicity of pyridine. Benzylpyridine prepared by the method of Tschitschibabin (21), and apparently a mixture of the α- and β-derivatives, was more toxic than any other pyridine derivative tested, except nicotine. Concentrations of about 0·2 per cent. killed 100 per cent. of the insects.

The toxicity of the pyridine ring can therefore be increased by hydrogenation, by the introduction of side chains and by fusion with one or more benzenoid rings. A fourth method is the attachment of a second pyridine ring as in the dipyridyls; these compounds are not dealt with in this paper but the work of Richardson and Smith (13, 14) shows them to be much more toxic than pyridine[1].

The Dependence of the Toxic Values of Insecticides on the Phase in which they are applied.

Tattersfield and Roberts (22) tested the insecticidal values of a few of the compounds dealt with in this paper, using wire-worms as the test-subject and applying the insecticide in the vapour phase in closed flasks

[1] Since this paper was handed in for publication, a full account of the work of these authors on the dipyridyls has appeared (*Jour. Agr. Res.* 1926, **33**, 597), in which the high toxicity of certain of these derivatives to insects is demonstrated.

under standard conditions. Although these conditions differ widely from those employed in our spraying technique, it is of some interest to make a general comparison of the kind of effects obtained, although it cannot be put on a quantitative basis.

Amongst the inorganic compounds, ammonia gas was, relative to other compounds, moderately toxic to wireworms, when its concentration was expressed in fractions of a gram-molecule per litre of air, and highly toxic when expressed in grams per litre of air. As a spray fluid, ammonium hydroxide was scarcely toxic to aphides at a concentration of 9 gm. per 100 c.c. Although the amount of ammonia falling on any individual aphid can only be very minute and will evaporate at a fairly rapid rate, it is of interest that, to an insect of the type of *A. rumicis*, ammonia should be comparatively harmless when applied in solution, whereas when applied as gas, it should have so profound an effect upon wireworms, commonly regarded as highly resistant insects.

Benzene, toluene, xylene are not active as insecticides (3) but the toxicity increases with molecular weight; this agrees with the type of results obtained by Tattersfield and Roberts for the effect of these substances in the vapour phase. There is, moreover, both in the liquid and vapour phases a definite increase in toxicity on substituting an NH_2 group in these rings. There is also in both cases an increase in toxicity produced by methylating the amino group of aniline.

The only other instance common to both investigations, in which direct comparisons can be made, is the case of pyridine, found by us to have a toxicity to *A. rumicis* of a very low order when used as a spray fluid and by Tattersfield and Roberts to be only moderately toxic as vapour to wireworms. As already noted however pyridine in the vapour phase has apparently a specific toxic action to mites and their eggs.

α-Naphthylamine and diphenylamine were non-toxic under the conditions of the experiments of Tattersfield and Roberts, whereas as sprays they are moderately toxic. Presumably the toxicity of these compounds in the vapour phase was limited by low volatility, and a prolonged exposure of the insects might have changed the category in which these compounds were placed.

Plot experiments.

A few substances found toxic in the laboratory experiments were tried on a larger scale on plots of broad beans badly infested with *A. rumicis*. Wetting and spreading was ensured by making up each spray fluid with 0·35 per cent. soft soap solution.

Tetramethylammonium chloride and sulphate were both tried under these conditions; they were completely effective in controlling the aphides at a concentration of 0·35 per cent. of the base, and there was no sign of injury to the plants. The sulphate also gave good results at a concentration of 0·125 per cent. of the base.

Some of the aromatic amines were also tried. α-Naphthylamine and mono-methyl-α-naphthylamine destroyed the aphides when used at concentrations of 0·4 per cent. and above, but also caused considerable damage and staining of the foliage. The latter effect was not shown by dimethyl-α-naphthylamine which however was slightly less effective as an insecticide.

The only pyridine derivative tested in this manner was benzyl-pyridine. The results bore out completely the figures obtained in the laboratory experiments, all the insects being killed by a spray fluid containing 0·1 per cent. of this compound. Moreover, no damage whatever was caused to the plants. Benzyl-pyridine is an oil without disagreeable properties and, if it could be prepared reasonably cheaply, it might find some use as a contact insecticide. Its high toxicity warrants further work on the subject.

Summary.

1. The toxicities to *Aphis rumicis* of certain aliphatic and aromatic amines and of some of the simpler nitrogen-heterocyclic derivatives have been quantitatively determined.

2. Tetramethylammonium hydrate and chloride are more toxic than the corresponding tetraethylammonium compounds. This is in keeping with the findings of Dale and his co-workers who have shown that tetramethylammonium has certain physiological effects similar to those of nicotine which are not shown by tetraethylammonium.

3. The aromatic amines, on the whole, show little insecticidal action. Aniline and most of the aliphatic anilines are only slightly toxic to *A. rumicis*. The substitution of aromatic groups in the amino group of aniline increases toxicity more than the substitution of aliphatic groups. There are interesting relationships in regard to toxicity among these compounds. For example, the following orders of toxicity were noted:

Phenylamine (aniline) < diphenylamine > triphenylamine.
Phenylamine (aniline) < benzylaniline > dibenzylaniline.
Benzylamine < dibenzylamine > tribenzylamine.

4. *o*-Nitraniline is one of the most toxic of the aniline derivatives.

5. α-Naphthylamine is more toxic than aniline. Substitution of various radicals in the amino group of aniline has a greater effect on the toxicity of the resulting compound than substitution of the same radicals in α-naphthylamine. α-Naphthylamine derivatives are more toxic than the corresponding β-derivatives.

6. Among the heterocyclic compounds, nicotine is highly poisonous to *A. rumicis*. The heterocyclic rings constituting the molecule of nicotine are much less toxic than nicotine itself; pyrrole and pyridine show comparatively slight insecticidal action. The order of toxicity of the simpler N-heterocyclic compounds runs:

Pyrrole < pyridine < picoline < lutidine < quinoline and isoquinoline < acridine.

7. Hydrogenation of pyridine and pyrrole increases their toxicity; piperidine is more toxic than pyridine and pyrrolidine than pyrrole.

8. Benzyl-pyridine is the most toxic pyridine derivative tested.

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ON THE CONTROL OF GLASSHOUSE INSECTS WITH CALCIUM CYANIDE

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During the past few years, as pointed out by Speyer and Owen (4), efforts have been made in America and Germany to develop a means of generating hydrocyanic acid gas by a method simpler than that of jar cyaniding, which has certain attendant disadvantages, including the necessity for separate weighings and measurings of sodium cyanide, sulphuric acid and water, the need for numbers of jars in large commercial houses, the labour involved and the limitations resulting from weather conditions. As a result of investigations in the United States (1), calcium cyanide containing 40–50 per cent. $Ca(CN)_2$ was manufactured, and after tests by Weigel (7) and others, this material has become widely used as a cheap and convenient source of hydrocyanic acid gas. Several grades of calcium cyanide, including dust and fine and coarse granules, are now available; the fine granular form recommended for glasshouse fumigation is like sharp sand and resembles basic slag in colour. With calcium cyanide no acid is required to generate the gas, and no mixing is necessary, for on exposure it slowly reacts to the moisture in the atmosphere and hydrocyanic acid is given off. Under glasshouse conditions the reaction continues for several hours and thus renders possible a long exposure with a low concentration, the type of fumigation recommended by Lloyd (2) for the control of the greenhouse white fly, *Trialeurodes vaporariorum* Westw. In May 1925, calcium cyanide was introduced into Great Britain, and since that date a large number of fumigations have been carried out in various parts of England (3), using this material as a source of hydrocyanic acid for the control of white fly, aphides and thrips, and the following is an account of the results so far obtained. I am indebted to Mr C. T. N. Wakely, B.Sc., and Mr A. Turner, A.R.C.Sc.I., N.D.H., for their help in conducting the fumigations, and to Mr F. Laing of the British Museum and Dr J. Davidson of Rothamsted, for identifying certain of the insects dealt with.

Methods.

In conducting the fumigations herein discussed, the cubic capacity of the glasshouse was first determined, and the calcium cyanide was measured by means of a graduated distributing funnel. The funnel used was provided with a lid and a tap, the former to prevent the escape of the fumes during application, and the latter to provide an easy means of running the calcium cyanide off. The fumigant was applied along the paths of the house and all ventilators closed and doors locked for the night. Next day the house was opened up early and after an hour or so examination for results was made.

In recording the results, where no live insects of the species against which the fumigation was conducted, could be found after careful search, 100 per cent. kill was recorded; where, in a heavy infestation, only odd active specimens could be found 90 per cent. kill was recorded. Where the kill was only partial, estimations were made in several parts of the glasshouse and the results averaged. When the pest was present in small numbers only, counts were made and the percentage figure obtained from them. In the case of fumigations for the control of white fly, where a number of fumigations were carried out at the same dosage, the lowest and highest estimated percentage kill figures are given to indicate the range of control obtained. In the case of the aphid control fumigations, the per cent. kill figures represent the average estimated kills from the fumigations at each dosage. While absolute accuracy is not claimed, the figures serve to indicate the degree of control obtained, particularly when taken in conjunction with the number of fumigations carried out.

Experiments on the control of white fly (Trialeurodes vaporariorum Westw.).

Fifty-four fumigations were conducted in glasshouses in various parts of Great Britain and under a variety of conditions, and in twenty-seven cases 90 per cent. kill or over was obtained on the adult insects. The following table indicates the dosages employed and the conditions as to temperature and humidity obtaining at the time of fumigation.

Plants fumigated included chrysanthemum, azalea, runner bean, cucumber, freesia, tomato and arum. In most cases the temperature varied between 55° and 70° F. at the commencement of fumigation and the relative humidity varied from 70–95 per cent. The lower dosages were employed when fumigating young plants such as seedling tomatoes

No. of fumigations	Dosage per 1000 cu. ft. (oz.)	Greenhouse temperature	Relative humidity %	Control (kill) %
2	$\frac{1}{12}$	62–71° F.	—	60
2	$\frac{1}{8}$	60–67° F.	90	70–80
4	$\frac{1}{6}$	54–70° F.	72–95	80
1	$\frac{1}{5}$	64° F.	79	80
11	$\frac{1}{4}$	58–67° F.	71–87	90–100
17	$\frac{1}{3}$	56–68° F.	84–87	50–100
11	$\frac{1}{2}$	52–70° F.	—	50–100
2	$\frac{2}{3}$	58–70° F.	—	100
4	$\frac{3}{4}$	59–77° F.	—	100

and cucumbers. In spring, when plants are usually soft and tender, they can only be fumigated with a low dosage, but under suitable conditions dosages as low as $\frac{1}{12}$ oz. per 1000 cu. ft. were found to keep the white fly in satisfactory check. The $\frac{1}{4}$ oz. dosage was employed for plants such as tomatoes in normal growth; $\frac{1}{3}$ oz. for chrysanthemums, and the higher dosages from $\frac{1}{2}$ oz. to $\frac{3}{4}$ oz. for mature tomatoes towards the end of the season, under abnormally dry conditions. Increased dosages were, of course, necessary in leaky houses. The moisture factor was variable; under very dry conditions it was necessary to damp the paths an hour or two before fumigation; in other cases the paths were so damp that satisfactory fumigations were not obtained until the cyanide was sprinkled on dry boards. Under average conditions in glasshouses, however, sufficient moisture was present for the satisfactory evolution of hydrocyanic acid gas from the calcium cyanide. Previous to fumigating open tanks were usually covered with sacks or boards. The calcium cyanide was distributed by means of a funnel provided with a tap to allow for the regulation of the flow, thus permitting even distribution throughout the greenhouses. Local conditions influenced results to a considerable extent, leaky houses, excess of moisture on the paths, low temperatures and high winds often being responsible for unsatisfactory results.

Experiments on the control of aphides.

Several species of aphides cause considerable injury in glasshouses, and in the course of these investigations at least five species have been encountered in various parts of England, all being more or less common and generally distributed. The following table lists the species and fumigating conditions, together with the dosages used:

	Dosage per 1000 cu. ft. (oz.)	Greenhouse temperature	Relative humidity %	No. of fumigations	Results (kill) %
Myzus persicae Sulz.	$\frac{1}{7}$	71° F.	73	1	80
	$\frac{1}{6}$	62° F.	79	2	70
	$\frac{1}{4}$	58–62° F.	83–89	3	90
	$\frac{1}{3}$	52–66° F.	83–94	8	98
	$\frac{3}{7}$	60° F.	89	1	90
	$\frac{1}{2}$	52° F.	—	1	100
Macrosiphum gei Koch.	$\frac{1}{7}$	60° F.	86	2	75
	$\frac{1}{6}$	65° F.	—	1	60
M. lineatum V. d. Goot.	$\frac{2}{5}$	55° F.	88	2	50
Myzus circumflexus Buck.	$\frac{1}{4}$	53–65° F.	83–89	5	78
	$\frac{1}{3}$	59° F.	68	1	80
	$\frac{1}{2}$	53–55° F.	88–90	2	90
Macrosiphoniella sanborni Gillette.	$\frac{1}{4}$	53° F.	—	1	100
	$\frac{1}{3}$	53–55° F.	—	2	100
Aphis rumicis L.	$\frac{1}{5}$	59° F.	89	1	90

When used under suitable conditions, with the temperature between 60° and 70° F., and relative humidity of 80–90 per cent., effective control was generally obtained with a dosage of $\frac{1}{5}$–$\frac{1}{3}$ oz. of calcium cyanide per 1000 cu. ft. In practice it was found most satisfactory to employ a low dosage and carry out two or three fumigations at about weekly intervals. *Myzus persicae*, which lives and breeds throughout the year under glasshouse conditions and infests a great variety of plants, is the species most commonly met with under glass. *Myzus circumflexus* occurs commonly on arums and is occasionally found on tulips, cyclamen and freesias, though most glasshouse plants may be attacked. According to Theobald (5), this species increases most rapidly from February to May, although it is found all the year under glass. *Macrosiphum gei* Koch. (*solanifolii* Ashm.) occurred on tulip in the fumigations recorded above. This species is known to attack sprouting potatoes (6). *Macrosiphum lineatum* V. d. Goot occurred commonly on chrysanthemums at Worthing and Cambridge in November 1926. *Macrosiphoniella sanborni* occurs on chrysanthemums out of doors from September to November and throughout the winter under glass. Under ordinary greenhouse conditions it is found that the species is easily controlled at a dosage of $\frac{1}{4}$ oz. per 1000 cu. ft., whereas the other species under similar conditions may need a higher dosage.

Experiments on thrips.

During the course of these investigations four species of thrips have been dealt with: *Heliothrips bicinctus* Bagn. on smilax, *Thrips tabaci* Lind. on smilax, arums and carnations, *Parthenothrips dracaenae* Heeg.

on Kentias and other palms and *H. haemorrhoidalis* Bouché. on tomatoes, arums and scolopendrium fern. Fumigations with calcium cyanide were found to be successful in killing adult thrips, but failed to destroy the immature forms. In the case of attack by *T. tabaci* on carnations, the insects, especially the adults, infest the opening blooms and, where vast numbers are found deep down amongst the bases of the petals and within the sepals, their feeding may almost bleach the petals. The hydrocyanic acid gas penetrated the flowers well and was found to destroy thrips even in such locations. *H. haemorrhoidalis* occurs fairly commonly on arums, and in one case in Guernsey infestation was so severe in a house of tomatoes as to make the grower consider destroying the plants and re-planting. Periodic fumigations were found to destroy the adults and gradually reduce infestation, and the plants commenced making cleaner growth shortly after the first fumigation. The control of both species on arums was effected with comparative ease, but with *H. bicinctus* on smilax four fumigations were necessary to effect satisfactory control.

The following table lists the dosages employed and the conditions obtaining:

Species of thrips	Plant	Dosage per 1000 cu. ft. (oz.)	Greenhouse temperature	Relative humidity %	Result (kill) %
Thrips tabaci	Carnation	$\frac{1}{3}$	60° F.	—	10
	,,	$\frac{1}{2}$	62° F.	—	*
	,,	$\frac{2}{3}$	59° F.	—	65
Heliothrips haemorrhoidalis	Tomatoes	$\frac{1}{4}$	59–60° F.	72–73	30–40
	Arums	$\frac{3}{7}$	60° F.	89	70
H. bicinctus	Smilax	$\frac{1}{4}$	59° F.	97	10–20†
Parthenothrips dracaenae	Kentia palms	$1\frac{1}{2}$	60° F.	—	90‡

* Partial control. † Many stupefied but revived later. ‡ Nymphs apparently affected.

Calcium cyanide fumigations and mealy bug control.

Though no experimental fumigations have been carried out where definite counts for mortality give an accurate idea of the amount of control obtained, the following experiments in Guernsey indicate that calcium cyanide will give satisfactory control of mealy bug on tomatoes and vines. Tomatoes grown in a glasshouse where *Pseudococcus maritimus* Erh. occurs as a pest each year, were found by the writer to be infested in April 1926, when the plants were about 2 ft. high. Two or three little clusters of the insects occurred on each plant and the grower proposed using calcium cyanide for the control of aphides which were also present. Weekly fumigations, using $\frac{1}{5}$ oz. per 1000 cu. ft., resulted in

comparative freedom from mealy bug after 8 weeks, and at the end of July the grower stated that he had never had so little trouble with this pest, which this year had caused no damage either to the plants or the crop.

In another glasshouse in Guernsey, weekly fumigations for the control of the mealy bug on vines were commenced, just at the end of the flowering period (May 25th, 1926), with a dosage of $\frac{1}{4}$ oz. of calcium cyanide per 1000 cu. ft. This dosage was used for several weeks and then increased to $\frac{1}{3}$ oz. per 1000 cu. ft.

The treatment proved highly satisfactory and the grower stated that, whereas in other years all the bunches of fruit were infested before the end of June, at the end of July 1926 he could not find a single infested bunch.

Cost of fumigation with calcium cyanide.

The calcium cyanide method of fumigation compares very favourably as regards costs with the older method of fumigation with sodium cyanide and sulphuric acid. The cost of fumigating a glasshouse of 40,000 cu. ft. at $\frac{1}{4}$ oz. dosage works out at 1*s.* 8*d.* plus the cost of labour, which is small, since the operator has merely to pour the calcium cyanide from the tin into a graduated funnel and read off the required amount. The funnel tap is then turned and the fumigant applied along the paths as the operator walks through the glasshouse.

SUMMARY.

Experiments on glasshouse fumigation with calcium cyanide in Great Britain during 1925–26 indicate that this material is a satisfactory source of hydrocyanic acid gas, and can be used to control a number of the usual pests infesting glasshouse plants. *Trialeurodes vaporariorum* Westw. was held in check with dosages of $\frac{1}{12}$–$\frac{1}{6}$ oz. per 1000 cu. ft. and effectively controlled with dosages from $\frac{1}{4}$–$\frac{3}{4}$ oz. per 1000 cu. ft. according to the conditions prevailing.

At least six species of greenhouse aphides were controlled with dosages varying from $\frac{1}{7}$ oz. to $\frac{1}{2}$ oz. per 1000 cu. ft.

Thrips, of which four species were dealt with, were more difficult to control, apparently only the adults being affected by the hydrocyanic acid gas. Dosages of $\frac{1}{3}$ to $1\frac{1}{2}$ ozs. per 1000 cu. ft. killed varying percentages of the adults and a series of fumigations were found to give satisfactory control.

Two instances are cited where continued fumigations with calcium cyanide apparently resulted in control of mealy bugs on tomatoes and vines in Guernsey.

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THE EFFECT OF SOIL STORAGE AND WATER CONTENT ON THE PROTOZOAN POPULATION

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As it has been conclusively shown that protozoa form a constant constituent of the soil population, and since it is known that their relationship to the other members of that population is an important one, it was decided to re-investigate the effect of certain external conditions on their multiplication and life processes. The present paper deals with the effect of soil storage and of the water content of the soil.

Soil Storage.

In microbiological studies it has been and still is a common practice to store soils for laboratory experiments in bottles or similar containers for varying periods of time. Within the past few years, however, it has become apparent that such a procedure produces such large changes in both the size and composition of the soil population that accurate experiments are impossible with soils so treated. Lipman (10) concludes that in soils kept in pots under greenhouse conditions the filling of the pots and the application of fertilisers involve a more intimate contact of the soil particles with atmospheric oxygen than is possible under field or garden conditions. This leads to an abnormal multiplication of the soil bacteria and to a consequent abnormally rapid oxidation of the organic matter. In the course of time the more readily decomposable portions of the organic matter become depleted and this is followed, in turn, by a decline in the numbers of bacteria. The numbers of the bacteria in his experiments all showed after a period of two months a large decrease.

Waksman (15) similarly found that fungi and bacteria in soil kept undisturbed in covered earthenware pots after the first few days showed a general falling off in numbers until the 174th day when the decrease was very slow. Eleven months after the soils had been bottled the numbers of micro-organisms did not change appreciably, a uniform balance of microbiological activity having been reached. Allison (1)

concludes that lack of aeration was a determining factor in causing decrease in numbers during storage of soil.

Fantham and Paterson (6) in their work on the South African soils showed that certain species of protozoa persist, though not all survive, in soil bottled for three years; and that the sequence of appearance of the protozoa in cultures is altered after storing; the ciliates from stored soil developing more rapidly in cultures than do those from fresh soil. Goodey (7) also obtained an abundant fauna of amoebae and flagellates from soils that had been stored for many years.

It is possible, however, that in all these cases the observers were dealing with the cysts of the protozoa, and that the active forms had disappeared during the period of storage. But since it is imperative, for certain kinds of experiments, that the soil shall be stored and placed under controlled laboratory conditions, it was decided to carry out investigations designed to discover a method by which the soil inhabitants were not injured by such treatment.

The species of protozoa which it was decided to consider were the common soil flagellates *Cercomonas crassicauda*, *Heteromita globosa*, *Oicomonas termo*, and the widely distributed soil amoebae *Dimastigamoeba gruberi*. At first the effect of placing sieved soils in bottles stoppered with cotton wool was tested, since in the past this has been a common method of conducting experiments. The bottles were kept stoppered for weekly intervals when they were opened to permit of taking a sample for counting purposes, and at the same time to adjust the water content which was maintained approximately at one half the water-holding capacity of the soil. Previously to removing the stoppers the soil was violently shaken in order to get uniform distribution, but at no time during the experiment was the soil taken out of the bottles. Counts of the protozoa and bacteria made at weekly intervals for five weeks showed a steady decrease in numbers. This diminution being in respect of the total numbers of the protozoa rather than in respect of the percentage of active forms, which remained fairly high throughout the experiment.

Since this might have been due to the artificial external conditions of the laboratory, a second experiment was carried out similar to the one already described, except that the bottles were buried up to the shoulders in a field, the tops being protected from heavy rain by small tin plates supported above them. As is seen, however, from Table I a decrease in the general population of the bottled soil still occurred even under these somewhat more natural conditions.

Table I.

Days	*Cercomonas* Total	*Cercomonas* % act.	*H. globosa* Total	*H. globosa* % act.	*Oicomonas* Total	*Oicomonas* % act.	*D. gruberi* Total	*D. gruberi* % act.	Bacteria in millions per gram	H_2O content
At start	172,000	99·1	55,400	98·0	55,400	97·1	18,300	96·5	19·7	21·2
7	9,720	64·4	2,400	0	4,800	63·8	1,200	0	16·4	22·5
14	6,600	0	1,160	0	2,300	0	256	0	13·4	23·8
21	8,500	0	4,700	82·5	2,300	75·2	420	95·6	11·9	23·6
28	6,700	0	1,700	80·3	2,300	27·8	600	0	10·0	24·5
35	4,700	0	2,400	0	1,900	0	1,200	84·2	8·1	23·7

A second experiment made a month later than that given in Table I but otherwise under identical experimental conditions gave similar results.

These two experiments are given as illustrations of many that were made at different periods of the year and with soils which had been variously manured. Since all the experiments gave similar results detailed reference to each is not necessary. There appeared to be two reasons for the effects observed:

(*a*) the effect of sieving;

(*b*) that the ratio of the surface area of the soil to its volume was insufficient for adequate aeration.

Experiments speedily eliminated the first possibility for no effect could be observed when sieved soil was compared with unsieved soil. It was decided therefore to concentrate on the second possibility.

Large quantities of soil were sieved and filled into bottles stoppered as before and also into earthenware pots such as are used for pot culture work, the pots having an internal diameter of 10 inches and a depth of 12 inches. The pots and bottles were kept outside and as far as possible protected from rain. The figures already given in Table I were obtained from the bottled soil: those given below in Table II are for the soil in the pots.

Table II.

Days	*Cercomonas* Total	*Cercomonas* % act.	*H. globosa* Total	*H. globosa* % act.	*Oicomonas* Total	*Oicomonas* % act.	*D. gruberi* Total	*D. gruberi* % act.	Bacteria in millions per gram	H_2O content
At start	172,000	99·1	55,400	98·0	55,400	97·1	18,300	96·5	19·7	21·2
7	8,200	27·4	2,030	50·0	5,720	0	4,500	64·0	18·6	24·2
14	13,200	91·0	1,180	0	6,700	82·4	290	0	21·4	20·5
21	61,000	0	13,400	87·0	13,400	63·0	1,780	91·9	11·3	25·2
28	250,000	97·1	10,300	87·8	3,300	64·5	1,060	82·4	24·3	26·3
35	420,000	95·0	250,000	96·1	350,000	97·5	690	0	16·5	20·4

Comparison of the two tables shows that the organisms in the pot soil behaved in a much more normal manner than those in the bottled soil. It is true that during the first week of the experiment there was a very marked drop in the numbers of all the species counted, but after this initial depression the population increased and there was an indication of the fluctuations in numbers which are associated with normal field soils. This was also shown by the bacteria. Owing to the pot not being as adequately protected from the weather as were the bottles, the water content of the former varied more during this experiment, but it is not thought that this is sufficient to account for the difference between the two sets of soil.

The final sets of the experiments made were with soil confined in earthenware pots of a shape giving a large surface area. These pots were shallow with a depth of 6 inches, but an internal diameter of 25 inches.

A series of five such experiments were done at different periods of the year, and it was found that after an initial depression, lasting for about three days, the numbers of both protozoa and bacteria remained normal and exhibited the fluctuations already referred to above.

The final series of these experiments were carried out towards the end of 1924 and since that date we have had an opportunity of testing the correctness of our conclusion, viz. that provided the surface area of the soil is large relative to its volume it is possible to carry out laboratory experiments on the micro-organisms of stored soils.

Repeatedly it has been necessary for us to investigate the effect of various treatments on the soil inhabitants; and when a small quantity of soil was needed it has been our practice to use a large Petri dish as the container. This procedure has been found to be quite satisfactory.

Effect of Water Content.

Regarding the effect of water content of the soil upon the activities of micro-organisms a certain amount of information has been obtained by early investigators.

Koch (8, 9) and Coppa (2) consider moisture to be the most important factor in the growth of soil protozoa. Koch's results indicate that the largest number of protozoa were recorded when the moisture was highest ($1\frac{3}{4}$ of optimum) and at the lowest ($\frac{1}{3}$ of optimum) protozoa did not become active. Waksman (14), on the contrary, states that "the soils with the optimum moisture gave in the main higher bacterial numbers ...than those containing full moisture holding capacity."

Cunningham (3) supports Koch's view, since he finds that the number

of protozoa is increased in the soil with a moisture content of 70 per cent. water-holding capacity and in saturated samples, where flagellates develop to the exclusion of amoebae. Waksman (13) finds that flagellates are present in soil with a moisture content of 14 per cent. which is too low for the development of the other groups. Sherman (12) also states that small flagellates will develop in soil of 11 per cent. water-holding capacity.

As the conclusions just cited were obtained from experiments in which either the direct method of counting was used or else where no distinction had been drawn between active and cystic stages of protozoa, it seemed desirable to re-investigate the problem using the methods elaborated in this laboratory (4); and counting the numbers of active and cystic forms of the same species of protozoa as were used for the storage experiments.

In the first set the soil was air dried and then moistened until it attained a water content equal to $\frac{1}{6}$ of the water-holding capacity. Owing to the drying all active protozoa were killed, but the animals in the cystic condition remained viable. Counts made at varying periods after the soil had been moistened would show whether there was sufficient water to allow of excystation and subsequent reproduction.

Two counts were made at intervals of $1\frac{1}{2}$ hours and 24 hours after the soil had been wetted. In only one case, that of *Cercomonas crassicauda*, did excystation and reproduction occur, the numbers of this animal increasing from 64,000 per gram to 230,000 in 24 hours.

The numbers of bacteria, which at the beginning of the experiment were 6·6 millions per gram, also showed an increase to 11·69 during the same period. Repetition of this experiment gave similar results.

It was therefore decided to carry out similar experiments with increasing water contents and at the same time to enumerate the numbers of active protozoa in addition to the total numbers. The results are given in Tables III, IV.

From these two experiments it would appear that soil having a water content of one-third or above its water-holding capacity provides a suitable medium for the excystation and reproduction of these four common soil protozoa and that at $\frac{1}{6}$ of the water-holding capacity only the species *Cercomonas crassicauda* is capable of survival.

It will also be noticed from the figures given in the tables that the organisms exhibited the same kind of fluctuations both in total numbers and the proportion of active and cystic forms as has been shown in earlier papers (4) to be characteristic of normal field soils.

Table III.

Soil air dried and water content brought to $\frac{1}{3}$ w.h.c.

	At start		After $1\frac{1}{2}$ hrs		After $4\frac{1}{2}$ hrs		After 24 hrs		After 48 hrs	
	No.	% act.	No.	% act.	No.	% act.	No.	% act.	No.	% act.
Cercomonas	230,000	0	95,000	88·4	64,000	43·7	230,000	85·6	140,000	83·7
H. globosa	21,000	0	21,000	85·6	3,600	50·0	44,000	95·9	5,100	67·5
Oicomonas	10,000	0	5,100	64·9	5,100	87·4	10,000	87·0	5,100	40·9
D gruberi	1,800	0	1,800	76·2	2,600	0	1,800	65·2	900	84·6

Table IV.

Soil air dried and water content brought to $\frac{1}{2}$ w.h.c.

	At start		After 3 hrs		After 6 hrs		After 24 hrs		After 72 hrs	
	No.	% act.	No.	% act.	No.	% act.	No.	% act.	No.	% act.
Cercomonas	44,000	0	95,000	37·8	230,000	84·3	420,000	85·9	420,000	85·9
H. globosa	41,000	0	44,000	0	15,000	80·2	140,000	83·6	140,000	96·2
Oicomonas	1,800	0	1,800	40·2	10,000	66·8	44,000	91·6	21,000	82·4
D. gruberi	7,300	0	10,000	20·1	15,000	26·6	21,000	87·6	30,000	87·6

In order to test how far these laboratory results are a true picture of what takes place in a field under normal environmental conditions, advantage was taken of a dry spell of weather in 1924 under which certain of the Rothamsted soils became deficient in water. Samples from the top four inches of the surface soil of one of the Barnfield plots were taken daily for three days and gave water contents of 6·4 per cent., 6·2 per cent. and 6·1 per cent. respectively, the water-holding capacity of the soil being 37·9 per cent. The results of the counts are given in Table V.

Table V.

	1st day		2nd day		3rd day	
	Total no.	% active	Total no.	% active	Total no.	% active
Cercomonas	64,000	94·2	15,000	67·5	44,000	87·9
H. globosa	2,600	0	2,600	0	2,600	0
Oicomonas	1,300	0	640	0	640	0
D. gruberi	1,800	0	900	0	1,300	0

These results are therefore in agreement with those obtained from laboratory experiments, and it is of interest to note that again the flagellate *Cercomonas* is capable of living in an active condition under conditions of extreme dryness.

Finally in another experiment soil was taken at various depths from two plots of Hoos Field: one of the plots was fallow, the other being cropped with wheat—Table VI.

Table VI.

	Fallow						Cropped					
	0–2 in.		2–4 in.		5–6½ in.		0–2 in.		2–4 in.		5–6½ in.	
Depth	Total	% act.	Total	% act.	Total	% act.	Total	% act.	Total	% act.	Total	% act.
Cercomonas	160	68·7	230	56·1	320	50·0	110	73·0	450	61·0	320	24·7
H. globosa	25	0	25	0	160	0	38	0	56	0	56	0
Oicomonas	25	0	79	0	25	0	25	0	25	0	0	0
D. gruberi	160	0	110	0	110	0	110	0	230	0	160	0
Water content	4·25		4·3		4·5		6·6		6·7		6·8	

Here again it is found that except in the case of *Cercomonas* activity was not possible for the protozoa at such low water content.

It would seem therefore that low water content of the soil constitutes a limiting factor for the development of protozoa, but that once the value rises above the limiting one, $\frac{1}{6}$ to $\frac{1}{5}$ the water-holding capacity, further increase in the amount of water does not have a very marked effect.

This view finds support in the results obtained by Cutler, Crump and Sandon (4) in 1922. The numbers of bacteria and of six species of protozoa were counted daily for a year. Throughout this period, except for the last nine days, the water content of the soil varied between 12 per cent. and 22 per cent., but no correlation could be found between the numbers of micro-organisms and the amount of soil water. During the last nine days of the experiment the soil gradually dried and there was an indication that this was resulting in a diminution in the number of active forms of the protozoa, and a consequent increase in the size of the bacterial population.

Sandon (11) in his work on the *Distribution of Protozoan Fauna of the Soil* discussing this question writes "that the statement frequently made...that moisture is the principal controlling factor, though very plausible, appears to be equally unfounded."

Provided that the moisture contents in question were above the limiting value, as was the case in the soils discussed by Sandon, this view would appear to be perfectly correct.

Summary.

1. Experiments are described showing that it is possible to conduct laboratory experiments on micro-organisms in stored soil, providing the surface of the soil is large relatively to its volume.

2. Experiments on the influence of moisture on the soil protozoa

show that active forms are found in soils containing a very low percentage of water.

3. The flagellate *Cercomonas crassicauda* was found to excyst and reproduce in air-dried soil brought to $\frac{1}{6}$ the water-holding capacity. The numbers of the bacteria also in this experiment nearly doubled their numbers in 24 hours.

4. Four common soil protozoa were found to behave normally in soil of $\frac{1}{2}$ and $\frac{1}{3}$ the water-holding capacity, which previously had been air-dried and then brought to the desired water content.

5. Field soils having water contents of 6 per cent. in one case and 4 per cent. in another corroborated the laboratory experiments. In all of these samples active *Cercomonas* were found, other protozoa being present only in the form of cysts.

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REVIEWS

The Insects of Australia and New Zealand. By R. J. Tillyard. Pp. xi + 560, with 44 Plates and 468 Text-figures. Sydney: Angus and Robertson, 1926.

Dr Tillyard is one of the most prolific and original entomological investigators of the present generation and it is seldom that he is unable to shed some fresh light or new interpretation on whatever problem he may be engaged. Those who look for originality in the present textbook will have little cause for complaint and, although intended primarily for students in Australia and New Zealand, it contains much that will appeal to a wider circle. The author's intimate knowledge of the insects of those two lands renders him well qualified for his task, and his wide knowledge of underlying principles has enabled him to produce a volume far above the level of a faunistic handbook.

The book has been wholly set up and printed in Australia and the result is excellent. The type is clear and there is a wealth of mostly original illustrations. Of the 1251 separate figures, 203 are coloured and are reproduced on plates. On the whole these coloured illustrations serve their purpose admirably and enable the species thus portrayed to be easily recognised. Great credit is due to Mrs Tillyard's skill in drawing the originals of so many of the plates. Another pleasing feature of this volume is the small number of misprints or other typographical errors.

The arrangement of the subject-matter is in the form of chapters; six of the latter are of the nature of generalities, while the remaining 24 chapters are each devoted to a separate order of insects. In the space at command it is only possible to comment here and there on the most important sections of the book. The general classification given in Chap. I offers little scope for criticism. Dr Tillyard divides the Insecta into 24 orders and wisely rejects the modern pluralistic tendencies which recognise as separate orders groups of no higher than sub-ordinal rank. The Anoplura are held to include the Mallophaga to which they are closely related: the author, however, has some doubts respecting the ordinal value of the Zoraptera which might quite well have been merged as a division of his Copeognatha. The evidence for the Zoraptera being social, rather than simply gregarious in habit, rests on very slender evidence. Chap. II deals with external morphology and can be recommended as one of the best introductions to the subject that is available. Exception, however, must be taken to the author designating the dorsal and ventral plates of the abdominal segments as tergites and sternites, rather than using the established terms terga and sterna respectively. On p. 10, with reference to segmentation, Dr Tillyard correctly defines the tergites as the separate sclerites of a tergum and the sternites as the sclerites of a sternum. Chap. III dealing with internal anatomy is naturally brief in a work of this description: the author is evidently not at his best in discussing this subject and not entirely accurate as regards matters of physiology. The subject of Life History forms Chap. IV and includes the essential facts of metamorphosis in so far as external changes are concerned. The immature forms of all metabolous insects are termed larvae, the author objecting to the word nymph, in the case of the Heimetabola, as being unscientific and there is a good deal to be said in favour of his view. Chaps. V, VI and VII are concerned with the Thysanura, Protura and Collembola respectively. With reference to the Thysanura, an error has crept in regarding the spiracles. The author gives "7 or 8 pairs" for this order whereas there are 9 pairs in the Machilidae, 10 pairs in *Projapyx* and the Lepismidae, and 11 pairs in certain species of *Japyx*. In the Campodeidae, Dr Tillyard states that spiracles are absent, but as a matter of fact, there are three pairs in *Campodea*. It is difficult to agree with his

reasoning with regard to the styli associated with the two hind pairs of legs being homologous with epipodites. The latter structures in Crustacea are borne on the coxopodite (sub-coxa in Machilidae) whereas in *Machilis* they are carried on the basipodite or coxa which suggests that they are the homologues of exopodites. Chap. VIII is devoted to the Plectoptera or May-flies and in this account the author has incorporated his recent researches on the venation which have shed fresh light upon its interpretation. The Odonata (Chap. IX) are discussed in quite a masterly fashion and the intricacies of their morphology very clearly explained. Chap. X provides an excellent guide to the Orthoptera which are abundantly represented in Australia. The Isoptera or Termites follow and with regard to these insects it is unusual to find an up-to-date writer adhering to the idea of the existence of neoteinic royal forms. Recent work demonstrates the probability that such castes are true adults in a brachypterous or an apterous state as the case may be. The Perlaria or Stone-flies (Chap. XIII) form a short section, but Dr Tillyard has made good use of the primitive forms of the Southern Hemisphere in what he has to say respecting these insects. The Copeognatha or Psocoptera (Chap. XVI) have come in for overhauling and are regrouped on more rational lines in the light of the author's own discoveries among the Lower Permian fossils. Passing on to Chap. XIX, the Hemiptera, there will be found an excellent account of an order of great economic importance. The stumbling-block of a satisfactory classification of the Fulgoroidea has been overcome with help from Mr F. Muir, and this feature is a valuable aid to the identification of the numerous species. The long chapter on Coleoptera is well justified since there are over 21,000 species of the order in the two lands concerned. The family Curculionidae heads the list in sheer number of forms and some very remarkable representatives are figured. Mention needs also to be made of the curious Longicorn *Tillyardia* and the interesting figure showing its remarkable pectinate palpi. Chap. XXII, on Hymenoptera, is unique in that the author has done good service in clearing up the greatly involved vein-nomenclature that has so long been a drawback. By applying the results of his studies on *Protohymen* and other types he has been able to simplify the Comstock-Needham system as applied to these insects and reduce its apparent anomalies into an orderly system. The rich Neuropterous fauna of Australia is well indicated in the chapter concerned and a number of little known larval types are figured. In treating of the Diptera, that group is subdivided into two main sections —the Nematocera and Brachycera. This somewhat heterodox arrangement is a welcome step over the more usual system and is in conformity with what was probably the primitive dichotomy of the order. The account of the ptilinum is somewhat obscure, and on p. 334 this organ is mentioned as lying within a cuticular "infolding" known as the frontal lunule. On this explanation it is difficult to imagine how so large an organ as the ptilinum can be evaginated from beneath what is apparently a hard sclerite, since no mention is made of the extensive ∩-shaped frontal suture through which the ptilinum is usually regarded as being expelled. On p. 365 it is stated that the frontal lunule and ptilinum are absent in the Syrphoidea. This is scarcely correct since the lunule is clearly demarcated in the Syrphidae and in some cases at least a functional ptilinum occurs, even though it may not persist. It appears from these remarks that the author may have confused the frontal lunule with the frontal suture which is wanting in the Syrphoidea or at most but meagrely developed. The chapter on Lepidoptera is perhaps one of the best ever written in a general textbook. The classification presented is a new one based on the work of Turner and is evidently designed to overcome the outstanding difficulty of grouping the large number of families into rational major divisions. The super-families adopted bring together, in many cases, in natural association closely related families and in this respect marks an advance on existing systems. The volume concludes with an excellent chapter on the fossil insects of Australia and New Zealand and a useful section on entomological technique. There is also a glossary of technical terms used and a very complete index of subjects and authors. The bibliographies are supplied at the end of each chapter concerned and include the essential literature, especially works of a taxonomic character.

A scholarly book of this character will prove a great stimulus to entomology in

the Antipodes. It is no small problem to survey a fauna comprising over 40,000 species, note practically all the important genera and diagnose over 400 separate families. Dr Tillyard has carried out his task with conspicuous success and the few errors here noted fade into insignificance in face of the essential merits of the whole work.

A. D. IMMS

The Aspergilli. By Charles Thom and Margaret B. Church. Baillière, Tindall and Cox, 1926. 22*s.* 6*d.*

The volume is divided into two portions, a general discussion and a detailed taxonomic study. Part one commences with a history of the genus, its morphology, and a discussion of the basis of description and classification. Culture methods are described and this leads to chapters on the physiology and biochemistry of the genus, more particularly enzymic and fermentative activities in relation to industry, and finally to Aspergilli in relation to animal disease. Part two deals with the nomenclature of the genus and its division into groups and contains full descriptions of individual species and doubtfully related forms. The volume concludes with a synoptical key to the Aspergilli, a list of sixty-six accepted species, eight and a half pages of references and an index. There are also fourteen text-figures and four plates of micro-photographs.

The book is somewhat uneven in treatment. The discussion of morphology and cytology might perhaps be improved, whilst on the other hand the chapters on physiology are quite masterly summaries. The value of the detailed taxonomic portion can only be judged by its use in practice, but the keys have been tried out on a number of soil Aspergilli in culture and found extremely convenient and critical. The bibliography is not arranged alphabetically but according to textual reference which somewhat detracts from its convenience. Also it is not so complete or up-to-date as might be desired. Apart from the use of the more unusual form of certain words such as "somber," "mollusk," "vigor" and "color" the language is extremely clear and unambiguous. It is a pity that the building of the volume is not stronger—of four copies that I have seen, pages are already loose in three, and this is a serious matter in a book that is essentially for constant use in the laboratory.

Any small criticisms, however, sink into insignificance beside the thanks that all micro-biologists must give to the authors for producing this monograph. No more difficult task could have been attempted, and that Dr Thom and Miss Church had the courage to assay it and carry it through to so successful an issue puts all students of the culture of micro-organisms in their debt.

WILLIAM B. BRIERLEY

Potato Varieties. By R. N. Salaman. Cambridge University Press, 1926. 25*s.*

This book is one of the most refreshingly original volumes that have appeared for some time. It is an intensely personal book, being in no sense an impartial and complete compilation of other people's views on the subject but the writing down by Dr Salaman of Dr Salaman's views, the results of Dr Salaman's investigations and the accumulated wisdom and experience of Dr Salaman's two decades of interest and research on the problems. There are very obvious lacunae in the book—indeed, even as a treatise on potato varieties, it is quite incomplete—and one can only feel glad that the author has resisted the temptation to fill in these gaps with second-hand knowledge for the sake of completion. Sooner or later they will come within the scope of Dr Salaman's personal work, for he has amazing energy and is almost omnivorous, and one looks forward to successive editions in which his personal observations on these issues also will be given. As it is the book is packed full of first-hand observations, acute suggestions and facts impossible to obtain elsewhere, all

dealing with topics selected by their appeal to Dr Salaman's interests, but of primary importance to all dealing with agricultural plants.

The wide-ranging character of the work will be appreciated by the chapter headings which are as follows: (I) The definition of a potato variety; (II) Historical sketch of the development of present-day varieties; (III) The life of a variety; (IV) The methods of producing new varieties; (V) The application of genetics to variety raising; (VI) The technique of hybridization; (VII) The classification of varieties; (VIII) Classificatory guide; (IX) Correlations or linkages; (X) Varietal differences of maturity; (XI) The yield or crop of the potato, and the genetic factors affecting the same; (XII) The yield or crop of the potato and the environmental factors affecting the same; (XIII) Yield trials; (XIV) Varietal differences in the production of ware; (XV) Varietal differences and their basis in chemical and histological characters of the tuber; (XVI) Differential histological features of the tuber; (XVII) Varietal resistance of disease; (XVIII) Virus diseases and varietal resistance to their infection; (XIX) Degenerative diseases of unknown origin; (XX) Varietal differences in respect to extremes of temperature; (XXI) The adaptation of varieties to special conditions of soil and climate; (XXII) Synonymity; (XXIII) The leaf index; (XXIV) Varietal descriptions; (XXV) Common employment of the varieties described; (XXVI) Descriptions of varieties with their history and synonyms. In addition, there are three appendices, one by Mr W. H. Parker on Yield Testing, a Bibliography, an Index and a detailed Contents. The mere citation of the contents headings shows the range of the book. Many of the chapters, however, are very short, two or three pages only, but others dealing with problems, where the author is more at home, are fuller, that on variety description for example, running to over 100 pages.

Hardly anyone, least of all the author, could carry out active experimental research on a congeries of such important problems for nearly two decades without accumulating a vast amount of valuable general experience. Very often such general experience is of a kind that cannot be included in formal research papers, especially in these days of compression, and usually therefore is lost to all other workers on the problems. In the present volume, however, the author has created his opportunity and produced a book which is at once notable largely because it is charged so richly with this general experience.

The vigorous personality of Dr Salaman runs through every page, at times in a most amusing manner. Thus his very first sentence rides roughshod over all philosophical adumbrations on problems of individuality and race, and, of one of the most contentious examples, asserts bluntly "no matter how it may have been produced, the fact must never be overlooked that a variety is in reality an individual and not a race." This intensely personal standpoint is maintained throughout and adds greatly to the enjoyment of reading the volume. The book is beautifully produced and illustrated by ten plates, of which three are in colour, and is a volume that should be readily available to all students and research workers in agriculture and botany.

WILLIAM B. BRIERLEY

ASSOCIATION OF ECONOMIC BIOLOGISTS

REPORT OF THE COUNCIL FOR THE YEAR 1926, PRESENTED TO THE ANNUAL MEETING, JANUARY 28TH, 1927

No important change in the style of the proceedings was made last year; six meetings devoted to papers and discussions were held at the Imperial College of Science to whose authorities the Council again desires to express its sincere thanks for the facilities afforded to the Association. These meetings were attended not quite so fully as might have been expected considering the number of members who live within easy distance of London, and the Council hope that greater enthusiasm may be shown in the ensuing years.

The Annual Provincial Meeting was to have been held at the Long Ashton Horticultural Institution but the industrial upheaval came along just when all the preparations for the meeting had been made, and the difficulty of railway transport was so great that it was decided to cancel the meeting. The Council desire to express their thanks to the Vice-Chancellor of Bristol University and to Prof. B. T. P. Barker and his colleagues for the trouble they had taken in preparing for the meeting and to record their hope that the invitation to visit this Institute may be renewed at some early date.

The Summer Meeting was held at the Research Station at Cheshunt and thanks to the excellent organisation of the Director, Dr W. F. Bewley, a very profitable and enjoyable day was spent there.

The Council grievously laments the loss it has sustained in the death of one of its members, Prof. F. W. Gamble, whose place has been taken by Dr J. W. Munro. During the year the Association has lost four members by death, ten members have resigned and ten new members have been added to the Roll which now stands at a total membership of 229.

The Council desires to make known that it has decided that steps shall be taken in the near future to register the Association under Section 20 of the Company's Consolidation Act of 1908, a step which in the opinion of the Council will be of benefit to the Association.

Papers read to the Association during the year 1926.

Jan. 29. Prof. V. H. BLACKMAN, Presidential Address: "Recent Work in Plant Pathology and its relation to Applied Botany."

Feb. 26. H. V. TAYLOR: "The Importance of Applied Botany to Modern Fruit Growing."

Mar. 26. LORD LOVAT: "Forestry in Relation to Science—The National Economic Aspects."

Professor A. W. BORTHWICK: "Forestry in Relation to Science—The Points of Contact of Forestry and Economic Biology."

Oct. 22. R. G. HATTON: "The Influence of the Root Stock on the Vigour, Productivity and General Behaviour of the Apple Tree."

Nov. 12. C. L. WITHYCOMBE: "The Sugar-Cane Frog Hopper Problem in Trinidad, a study of its Factors and Principles of Control."

Dec. 10. A. J. RIKER: "Studies of Crown Gall and Wound-overgrowths on Apple Nursery Stocks."

H. WORMALD: "Crown Gall with reference to its occurrence on Fruit Stocks in this Country."

REPORT OF THE HON. TREASURER FOR THE YEAR 1926, PRESENTED TO THE ANNUAL MEETING, JANUARY 28TH, 1927

The annual statement of the accounts of the Association for the year ending December 31st, 1926, is set out on p. 261. It has been our custom in the past to meet the expenses of the previous year's volume of the *Annals of Applied Biology* out of the revenue of the current year. This has been inevitable owing to the lateness in the appearance of the last part of the current volume for a number of years in succession. For the last two volumes this disadvantage has been largely eliminated and it is hoped in the near future to pay the expenses incurred by each volume out of the revenue of its own year. In order to make this adjustment it will be necessary to pay for two volumes out of a single year's income for one year only, but once this is accomplished the recurrence of the difficulty is not anticipated.

During the year 1926 current subscriptions amounted to £252. 14*s*. 2*d*. as compared with £220. 9*s*. 0*d*. for the previous year. Special efforts have been made to urge prompt payment of the annual contribution to the Association and the response thereto has left little cause for complaint. At the same time a great advance has been made in the payments of arrears, £54 has been received under this category as compared with only £7. 10*s*. 0*d*. for 1925.

The working expenses of the chief charge on the Association, viz. the *Annals of Applied Biology*, have been considerably lower for vol. XII than for the volume for the previous year. This reduction is entirely due to an increase in revenue derived from the sale of that volume and of back volumes and parts. The amount required to meet the publishers' charges for vol. XII is £126. 11*s*. 9*d*. as compared with £303. 1*s*. 0*d*. for vol. XI. Allowing for the cost of vol. XIII which is to be met out of our 1927 revenue, our assets exceed our liabilities by £622. 3*s*. 9*d*., of which £574. 2*s*. 5*d*. are represented by cash as is shown in the balance sheet.

A. D. IMMS,
Hon. Treasurer.

TREASURER'S STATEMENT FOR THE YEAR ENDING DECEMBER 31ST, 1926

CASH ACCOUNT.

Cr	£	*s.*	*d.*	*Dr*	£	*s.*	*d.*
Jan. 1. Cash at Bank . .	70	8	6	Postage	6	5	6
Dec. 31. Subscriptions:				Stationery and minor printing	8	16	11
A. Current . . .	252	14	2	Treasurer	136	17	8
B. Arrears . . .	54	6	0	Secretaries	6	19	6
C. Advances . .	12	10	0	Balance at Bank	71	13	3
Entrance Fees . .	4	4	0	Placed on Deposit	200	0	0
Contributions to cost of papers in *Annals*	28	12	6				
Bank Interest . .	7	17	8				
Total	£430	12	10	Total	£430	12	10

BALANCE SHEET.

Liabilities.	£	*s.*	*d.*	Assets.	£	*s.*	*d.*
Subscriptions in advance . .	12	10	0	Current a/c	71	13	3
Liability on *Annals*, vol. XIII	408	15	8	Deposit a/c	430	0	0
Excess of assets over liabilities	622	3	9	Subscriptions two years or less in arrears and considered good	5	0	0
				National Savings Certificates .	481	5	0
				Estimated value of stock of *Annals of Applied Biology* with publishers	55	11	2
Total	£1043	9	5	Total	£1043	9	5

A. D. IMMS, *Treasurer.*

We have examined the Treasurer's statement of expenditure and receipts and have found it correct. We consider that the above balance sheet correctly represents the position of the Association.

C. T. GIMINGHAM.
GEO. H. PETHYBRIDGE.